Metabolic connections to life history in fishes

by

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Abstract

Metabolic rate is often assumed to set the pace of life histories because organisms depend upon the energy acquired through metabolism for survival, growth, and reproduction. However, key links between metabolic rate, morphology, and ecology remain unexamined. First, I examined the energetics behind brain size in the blacktip shark (*Carcharhinus limbatus*) using gill surface area as an integrated correlate of metabolic rate. Both brain mass and gill surface area increased with body mass throughout ontogeny and individuals with larger brains for their body mass also had larger gill surface areas. Second, I asked whether life history traits explained variation in resting metabolic rate across fishes and found that only growth performance, which encompasses the trade-off between growth and maximum size, explained variation. Collectively, this work illustrates the importance of energetic trade-offs and emphasizes the need for empirical tests of assumptions and an integrated view of physiology and ecology.

Keywords: allometric scaling; direct metabolic constraints; expensive brain; Gill Oxygen Limitation Theory; Metabolic Theory of Ecology; temperature-size rule

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Chapter 1.

Introduction

Confronted with the complexity of nature, yet limited time and resources, ecologists search for broad patterns and processes that can help to explain how organisms survive, grow, and reproduce in their environments, and then use this knowledge to direct conservation efforts (Reynolds 2003, Dulvy et al. 2014, Juan-Jordá et al. 2015). For example, for many fish species there are insufficient data for traditional assessments of population status and extinction risk, impeding management and conservation (Costello et al. 2012, IUCN 2017). Indeed, of the Chondrichthyans assessed by the International Union for the Conservation of Nature, almost half (46.8%) are categorized as Data Deficient (Dulvy et al. 2014, IUCN 2017). However, there are a striking number of traits that correlate with each other and produce patterns that may prove useful. Life history traits group with each other so that organisms' life history strategies can be described by where they fall along a fast-slow continuum (Hutchings 2002, Bielby et al. 2007, Juan-Jordá et al. 2013, Healy et al. 2019). Body size and metabolic rate have been shown to correlate with each other, as well as with many life history traits (Kleiber 1932, White and Seymour 2004, Speakman 2005, Furness and Speakman 2008). Furthermore, morphological traits like brain size and gill surface area have also been linked to both metabolism and life histories (Hofman 1983, Tsuboi et al. 2015, Gillooly et al. 2016, Bigman et al. 2018). Thus, understanding the interrelationships between metabolism, morphology, and life histories may provide a useful tool for predicting relationships across taxa and could potentially offer simple, trait-based approaches to support the development of ecological risk analyses (Reynolds 2003, Thygesen et al. 2005, Abelson 2016).

Being able to predict life history strategies from other, easier-to-measure traits could allow insight into individual, population, and community ecology, and allow estimates of extinction risk. Species can be prioritized for future research and management based on their sensitivity to extinction, which is a function of both intrinsic sensitivity and extrinsic exposure to a threatening process (Reynolds 2003, Dulvy et al. 2004, 2014). Species with slower population growth are intrinsically more sensitive to

threatening processes such as overfishing. However, because estimates of population growth are scarce, researchers often use life history traits that correlate with population growth rates to estimate intrinsic sensitivity (Denney et al. 2002, Reynolds 2003, Hutchings et al. 2012). Therefore, intrinsic sensitivity is typically inferred using size-related and age-related life history traits like maximum size or generation length (Dulvy et al. 2014, Juan-Jordá et al. 2015). But both population growth rates and many life history traits are difficult to measure, because they require time- and specimen-intensive sampling and detailed datasets. Thus, an alternate way to infer intrinsic sensitivity would be extremely valuable as it could fill some of the knowledge gaps for data-poor species as well as improve our understanding of an organisms' life history and ecology.

Metabolic rate is thought to influence an organism's ecology by governing how resources are collected and allocated among competing functions, acting as an intermediary between physiological processes and ecological patterns (Brown et al. 2004, Sibly et al. 2012). Metabolic rate is the rate at which organisms take in resources, convert those resources into energy for maintenance, growth, activity, and reproduction, and excrete waste back into their environment. Importantly, animals must balance the cognitive benefits of a large brain with the high energetic demands of neural tissue (Armstrong 1983, Hofman 1983, Isler and van Schaik 2009). Additionally, as individuals have finite resources and energy to allocate among competing functions of survival, growth, and reproduction, life history strategies are thought to arise as an optimization of these trade-offs that maximizes lifetime reproductive success (Law 1979, Stearns 1989, Healy et al. 2019). Suites of life history traits cluster together so that an organism's life history can be described by where it sits along a fast-slow axis (the 'pace of life'), with organisms that grow more slowly, mature later, live longer, and have a larger maximum body size on the 'slow' end of the continuum, and organisms with the opposite suite of traits on the fast end (Reynolds 2003, Bielby et al. 2007, Juan-Jordá et al. 2013, Healy et al. 2019). Metabolic rate is thus often assumed to influence brain size and determine where an organism sits along the fast-slow continuum of life histories, with organisms with higher metabolic rates supposedly having larger brains for their body size and sitting towards the 'faster' end of the slow-fast life history continuum (Armstrong 1983, Hofman 1983, Brown et al. 2004, Sibly et al. 2012). However, although there is a strong theoretical basis for the links between metabolic rate, brain size, and life history, many of these connections have not been empirically tested, particularly in ectothermic taxa.

Despite the potential utility of metabolic rate and metabolically important traits for understanding and even predicting life histories, there is still substantial uncertainty surrounding the interrelationships between these traits, particularly in ectothermic taxa. Metabolic rate may vary with brain size as brains are energetically expensive organs (Mink et al. 1981). For instance, given the energetic cost of maintaining brain tissue, organisms with a large brain for their body size may also require the capacity for higher energy turnover to sustain their brain. While metabolic rate has been positively related to brain size across endothermic species, we still do not know whether this relationship applies within ectothermic species (Armstrong 1983, Hofman 1983, Isler and van Schaik 2009). Additionally, metabolic rate may vary across species with different life histories, but previous work on endotherms has produced conflicting results as to whether metabolic rate and many life history traits are related across species (e.g. Magalhães et al. 2007, Furness and Speakman 2008).

In this thesis, I investigate how brain size and life history relate to metabolism in fishes. My aim is to answer two key questions: first, how does brain size relate to metabolic rate in the blacktip shark (*Carcharhinus limbatus*; Chapter 2); and second, do life histories explain variation in metabolic rate across fish species (Chapter 3). Chapter 2 of my thesis uses gill surface area as an integrated correlate of energy use and oxygen demand to provide a close look at how brain size relates to metabolic rate over longer time scales that match the trajectory of changes in blacktip shark brain size. Chapter 3 of my thesis is a meta-analysis of resting metabolic rate and various measures of life history across 104 species of bony and cartilaginous fishes to explore the connection between metabolic rate and life histories. Finally, in the concluding chapter, I synthesize and examine my findings in the context of metabolic ecology and discuss the potential use of measurements of metabolic rate and morphological traits to enhance our understanding of life histories and, in turn, to direct management and conservation efforts.

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Chapter 2.

Gill surface area provides a clue for the respiratory basis of brain size in the blacktip shark (*Carcharhinus limbatus*)

2.1. Abstract

Brain size varies dramatically, both within and across species, and this variation is often believed to be the result of trade-offs between the cognitive benefits of having a large brain and the energetic cost of sustaining neural tissue. One potential consequence of having a large brain is that organisms must also meet the associated high energetic demands. Thus, a key question is whether metabolic rate correlates with brain size. However, using metabolic rate to measure energetic demand yields a relatively instantaneous and dynamic measure of energy turnover, which is incompatible with the longer evolutionary timescale of changes in brain size within and across species. Morphological traits associated with oxygen consumption, such as gill surface area, can potentially serve as integrated correlates of energy use and oxygen demand. This allows us to evaluate whether evolutionary changes in brain size are matched by changes in longer-term energy availability. To assess this, we ask how brain size relates to gill surface area in the blacktip shark *Carcharhinus limbatus*. Firstly, we examine whether the allometric slope of brain mass (i.e., the rate that brain mass changes with body mass) is lower than the allometric slope of gill surface area across ontogeny. Secondly, we test whether gill surface area explains variation in brain mass, after accounting for the effects of body mass. We found that brain mass and gill surface area both had positive allometric slopes, with larger individuals having larger brains and larger gill surface areas compared to smaller individuals, but that the allometric slope for brains was lower than the gill surface area allometric slope, consistent with our prediction that the allometric slope of gill surface area could pose an upper limit to the allometric slope of brain mass. Finally, after accounting for body mass, individuals with larger brains tended to have larger gill surface areas. Together, our results provide clues as to how fish may evolve and maintain large brains despite their high energetic cost, suggesting that blacktip shark individuals with a large gill surface area for their body mass may be

able to support a higher energetic turnover, and, in turn, a larger brain for their body mass.

2.2. Introduction

Animals must balance the selective benefits of greater behavioral complexity and cognitive function with the high energy demands of having a large brain for a given body size. Having a large brain for a given body size has been associated with cognitive capability in a variety of vertebrate groups (Sol et al. 2008, Maklakov et al. 2011, Horschler et al. 2019), although some other studies challenge these findings (Turschwell and White 2016, Fichtel et al. 2020). Although many factors likely influence the evolution of brain size, large brains for a given body size have been linked to both habitat and social complexity across fish species, with some studies suggesting that that the cognitive requirements for living in spatially complex habitats such as reefs might have influenced the evolution of brain size, while others suggest that social behaviors and intra- and interspecific (i.e., within a species and across different species) interactions may also play a role (Bauchot et al. 1977, Yopak et al. 2007, Gonzalez-Voyer et al. 2009). Experimentally, guppies (*Poecilia reticulata*) selected for larger brains for their body size outperformed small-brained individuals in both a numerical learning assay and a spatial learning task (Kotrschal et al. 2013, 2015). Consequently, there appear to be clear benefits of – and potentially a high selection pressure for – large brain size relative to body size. On the other hand, large brains come at an energetic cost. The brain requires considerably more energy per unit weight than most other organs, accounting for at least 2–8% of resting oxygen consumption in vertebrates (Mink et al. 1981). Additionally, fishes (like reptiles and amphibians) are thought to grow throughout their lives and exhibit lifelong neurogenesis and brain growth and thus must meet the costs of both the growth of new brain tissue and the maintenance of existing brain tissue (Leonard et al. 1978, Zupanc 2006, Maruska et al. 2012). Therefore, despite the potential cognitive benefits of having a large brain, the energetic requirements of maintaining such a brain could constrain brain size evolution, particularly in ectotherms (Isler and van Schaik 2009).

Evolving a large brain for a given body size may require a decrease in other energy requirements (as suggested by the energy trade-off hypothesis), or an increase in overall energy turnover (as suggested by the direct metabolic constraints hypothesis;

Armstrong 1983, Hofman 1983, Isler and van Schaik 2009). Although data is scarce, larger brains for a given body size have been found in fish populations and species with higher oxygen availability or higher metabolic rates. For instance, populations of mormyrid fishes in well-oxygenated waters had larger brains than those in oxygen-stressed environments (Chapman and Hulen 2001) and, within fish species, brain mass appears positively related to metabolic rate after controlling for body mass, despite a lack of statistical significance (Sukhum et al. 2019). On an interspecific scale, brain mass has been correlated with water depth (a proxy for metabolic rate across fish species, Iglesias et al. 2015) although other studies have found no relationship between the two (Tsuboi et al. 2015). Additionally, brain mass correlates positively and significantly with resting metabolic rate across mormyrid fish species, even after controlling for evolutionary history and body mass (Sukhum et al. 2016). However, metabolic rate (i.e., oxygen consumption per unit time) is a relatively instantaneous and dynamic measure of energy use, whereas brain size evolution is likely shaped by longer-term energetic conditions (Carlson et al. 2004). Thus, a morphological trait that can be used as an integrated correlate of metabolic requirements on an appropriate timescale would improve our understanding of the energetic and oxygen requirements associated with having a large brain.

For most fishes, oxygen supply for aerobic metabolism is facilitated by the diffusion of oxygen across gills, resulting in a close relationship between gill surface area and metabolic rate (Hughes 1966, Wegner 2011). Fick's second law of diffusion provides the framework for the role of gill surface area in metabolic oxygen supply capacity and shows that a larger respiratory surface area augments oxygen uptake (Fick 1855 in Gillooly et al. 2016). Indeed, gill surface area is a metabolically important trait in fishes, and this has been shown on both intraspecific and interspecific scales (Hughes 1978, Wegner et al. 2010). Intraspecific comparisons of ontogenetic allometries show that metabolic rate and gill surface area scale at similar rates with body mass (De Jager and Dekkers 1975, Hughes 1978). Across species, fishes with higher metabolic rates have larger gill surface areas at a given body mass, and respiratory surface area and oxygen consumption also scale at the same rate with body mass across fishes and other vertebrates (Bigman et al. submitted, Hughes 1966, Gillooly et al. 2016). Thus, investigating gill surface area in tandem with brain size will improve our understanding of the energetic basis of brain size.

In addition to exhibiting lifelong neurogenesis, fishes grow throughout their lives (i.e., indeterminately) with both brain mass and gill surface area changing with body mass throughout ontogeny, so both brain mass and gill surface area must be studied in an allometric context and any comparison between brain mass and gill surface area must also account for body mass (Bigman et al. 2018, Laforest et al. in press, Lisney et al. 2017). Although explanations for the rate at which brain mass changes with body mass (i.e., the allometric slope of brain mass) within a species and across ontogeny are rare, some explanations have been proposed for allometric slopes across species. Because both brains and bodies are three-dimensional, brain mass may be expected to increase one-to-one with body mass (i.e., have an allometric slope of 1) according to simple geometry, however, this has been demonstrated not to be the case. For many years, the allometric slope of brain size was thought to be $^{2}/_{3}$, potentially due to brain mass innervating a two-dimensional body surface that increases with three-dimensional body mass (Jerison 1973 in Harvey and Bennett 1983). However, findings of an allometric slope of approximately $^{3}/_{4}$ across mammal species then led some to speculate that brain size was matched to metabolic rate based on the similarity of allometric slopes between brain mass and metabolic rate (metabolic rate was also commonly believed to scale with body mass with a slope of $^{3}/_{4}$; Martin 1981, Armstrong 1983, Hofman 1983). Recent interspecific analyses that correct for shared evolutionary history, as well as intraspecific studies on the allometric scaling of brain mass, have found slopes much lower than these predictions, tending to range from 0.4 to 0.6 though there are few suggestions as to why this might be (Bauchot et al. 1976, Tsuboi et al. 2018, Yopak et al. 2019). Theoretically, the allometric slope of brain mass could have an upper limit imposed by the energetic and oxygen requirements of brain growth and maintenance. Because gill surface area is the surface over which the oxygen necessary for aerobic metabolism diffuses and is expected to change with body mass with a slope of $^{2}/_{3}$ to 1 (Wegner et al. 2010, Bigman et al. 2018), the allometric slope of gill surface area with body mass may necessitate a shallower allometric slope of brain mass with body mass. This may explain the variation in allometric slopes seen both within and across species, since the allometric slope of brain mass would only have an upper limit (set by the allometric slope of gill surface area or metabolic rate) rather than exactly match a specific allometric slope value such as $^{2}/_{3}$ or $^{3}/_{4}$ (Karbowski 2007).

Chondrichthyans present a valuable opportunity to study the allometric relationships between brain mass and gill surface area. Firstly, ectothermic animals can shed light on the energetic costs of brains without the additional energetic costs of thermoregulation, yet studies of ectotherms are rare compared to those of endotherms. Secondly, chondrichthyans are known to possess relatively large brains in comparison to other ectothermic vertebrates (Myagkov 1991, Lisney and Collin 2006, Tsuboi et al. 2018). Thirdly, chondrichthyans, like other fishes, are thought to grow throughout their lives and exhibit lifelong neurogenesis and brain growth making studying their brains in an allometric context particularly important (Leonard et al. 1978, Zupanc 2006, Maruska et al. 2012). Fourthly, chondrichthyans are both the first extant jawed vertebrates (i.e., gnathostomes) and also the first group to exhibit the fundamental and highly homologous vertebrate brain plan (Yopak 2012). But, despite their basal place in vertebrate evolution, chondrichthyan brains have been poorly studied in comparison to other vertebrate groups, with brain mass data only available for about 16% of all currently described chondrichthyan species and even less is understood about intraspecific variation in brain size in chondrichthyans (Yopak 2012). We chose to study the blacktip shark *Carcharhinus limbatus* from the western Atlantic Ocean. Although there are many studies on the ecology of the blacktip shark, to our knowledge there is only one estimate of their brain mass in the literature, likely from a single individual (Myagkov 1991, Carlson et al. 2006).

Here, we explore how brain mass relates to gill surface area in the blacktip shark. We first ask whether the allometric slope of brain mass is lower than the allometric slope of gill surface area across ontogeny. Secondly, we ask if blacktip shark individuals with a larger brain also have a larger gill surface area for their body mass. We predict that both brain mass and gill surface area will have positive allometric slopes, but that the rate that brain mass changes with body mass will be lower than the rate that gill surface area changes with body mass because the energetic and oxygen requirements of the brain must be lower than the oxygen supplied over the gill surface area. Additionally, we predict that individuals with larger than expected brains for their body mass will also have a larger gill surface area.

2.3. Methods

2.3.1. Sample collection

We received specimens of blacktip shark that were opportunistically collected in August 2017 from fisheries-independent and fisheries-dependent trawl and longline surveys in the northeastern Gulf of Mexico near Panama City, Florida (n = 18) and the southern Atlantic Bight, off South Carolina and Georgia (n = 10). For each specimen, fork length (cm) and total length (cm) were measured. When possible, body mass (g) was also measured; otherwise, mass was estimated using previously published population-specific fork length-weight regression equations (SEDAR 29, 2012). After capture, the head was removed, the chondrocranium of each specimen was opened to allow the brain tissue to fix, and specimens were placed in 10% neutral-buffered formalin for later processing. Following previous work, which assumes minimal shrinkage due to fixation, neither brain mass nor gill surface area were corrected for such shrinkage (Yopak et al. 2007, Wootton et al. 2015).

2.3.2. Brain mass measurement

Total brain mass was measured in a manner consistent with previous methods following Yopak et al. (2007). Each brain was excised from the chondrocranium and separated from the spinal cord caudal to the posterior tip of the fossa rhomboidea in the region of the first complete cervical spinal nerve (Yopak et al. 2007, Lisney et al. 2017). The meninges, blood vessels, and connective tissue were removed, and the cranial and sensory nerves were transected to within 1 mm of their base. Each brain was blotted and weighed to the nearest 0.01 g (ScoutProScale).

2.3.3. Gill surface area measurement

Total gill surface area was estimated according to Muir and Hughes (1969) and Hughes (1984):

$$A = L_{fil} * 2n_{lam} * A_{lam},$$

where A is gill surface area, L_{fil} is the total length of all gill filaments on both sides of the head, n_{lam} is the lamellar frequency (i.e., the average number of lamellae per unit length on one side of a filament, doubled to account for both sides of the filament), and A_{lam} is the mean bilateral surface area of an individual lamella (Muir and Hughes 1969, Hughes 1984, Wegner 2011). This standard method of measuring gill surface area was chosen to make our results comparable to other gill surface area estimates for elasmobranchs and other fishes (Wegner et al. 2010, Wootton et al. 2015, Bigman et al. 2018). Dissections were performed using a dissecting scope fitted with a digital camera (either Zeiss Stemi 2000-C with a Lumenera INFINITYLite camera or Meiji Stereo EMZ-8TR with a MoticCam 5+). Because gill surface area is symmetrical and either side can be used for measurements, we used either the right or left side depending on the condition of the filaments (Wegner 2011). For all but one individual, gill surface area was measured on the right side of the head; for the remaining individual, gill arches were only available for the left side of head. For a more detailed description of the dissection procedure see Bigman et al. (2018).

It was not possible to measure brain mass and gill surface area on all individuals. For 15 individuals, both brain mass and gill surface area were measured; for a further nine individuals, only brain mass was measured and for a further four individuals, only gill surface area was measured. Though this did not affect the body mass range over which gill surface area was measured (750 – 30,043 g), it resulted in a decreased body mass range for which brain mass could be measured (750 – 12,977 g, Table 2.1).

2.3.4. Statistical analyses

Is the allometric slope of brain mass lower than the allometric slope of gill surface area?

To test whether the allometric slope of brain mass was lower than the allometric slope of gill surface area, we fit two linear models, one for the relationship of brain mass and body mass (n = 24), and one for the relationship of gill surface area and body mass (n = 19). As samples came from two different locations, the Gulf of Mexico and the south Atlantic Bight, we assessed whether the allometric relationships of brain mass and gill surface area differed between the locations using location as a fixed factor and including an interaction with body mass (Table A.1). This parameterization allows for the

estimation of location-specific allometric slopes and standardized intercepts which were then compared between locations by assessing the overlap of the 95% confidence intervals (CIs). As we found little effect of location for this small and biased sample size, we pooled the data for subsequent analyses (Figure A.1, Table A.2). To be consistent with previous work, we used linear regression on $\log_{10}$-transformed data for both brain mass and gill surface area allometric relationships (Chapman and Hulen 2001, Salas et al. 2015, Bigman et al. 2018). On a $\log_{10}$-transformed scale, the intercept is estimated at 1 g of body mass, which lies far outside the range of body masses for the specimens in this study. To avoid this extrapolation of the intercept, which can lead to a correlation between intercepts and slope, we centered the body mass data around 2,000 g (approximately the median of our specimen size range; Quinn and Keough 2002, Bigman et al. 2018). Thus, the intercept is estimated at a more meaningful body mass (2,000 g) and is termed the 'standardized intercept'. Importantly, the intercept can be centered on any value, and thus can be interpreted biologically as the gill surface area or brain mass at a given body mass (Bigman et al. 2018). All statistical analyses were performed in R v. 3.6.1 (R Core Team, 2019).

Do individuals with larger brains for their body mass also have larger gill surface areas?

To determine whether individuals with a larger brain also have a larger gill surface area after accounting for body mass, we parameterized two linear models using data from individuals for which both gill surface area and brain mass were measured (n = 15). In both models, brain mass was the response variable, but in the first model only body mass was an explanatory variable while in the second model both gill surface area and body mass were explanatory variables. Both explanatory and response variables were $\log_{10}$-transformed, and body mass was centered to 2,000 g, as above. To identify the model that provided the best fit to the data, we compared these two candidate models using the corrected Akaike information criteria (AICc) for small sample sizes, which penalizes models for their number of estimated parameters, with smaller AICc values indicating a better fitting model (Hurvich and Tsai 1989, Burnham and Anderson 2002). The weight of evidence for any given model out of those tested was measured by its Akaike weight (w_i), the relative likelihood of the model divided by the sum of the likelihoods of all other models.

2.4. Results

Gill surface area estimates ranged from 2,463 to 58,205 cm^2 over the body mass range of 750 to 30,043 g (n = 19) while brain mass ranged from 6.02 to 21.23 g over the over the body mass range of 750 to 12,977 g (n = 24, Table 2.1).

Is the allometric slope of brain mass lower than the allometric slope of gill surface area?

Yes, the allometric slope of brain mass was positive, but lower than the allometric slope of gill surface area. The rate at which brain mass changed with body mass was 0.45 (95% CI: 0.41–0.49, Figure 2.1A) while the rate at which gill surface area changed with body mass was 0.90 (95% CI: 0.81–0.99, Figure 2.1B, Table 2.2). For instance, for a doubling (i.e., a 100% increase) in body mass from 2,000 g to 4,000 g, brain mass increased by about 37% while gill surface area increased by about 86%. Thus, a 2,000 g individual was predicted to have around an 8.41 g brain and a 5,282.82 cm^2 gill surface area, while a 4,000 g individual was predicted to have around an 11.49 g brain and a 9,848.31 cm^2 gill surface area.

Do individuals with larger brains for their body mass also have larger gill surface areas?

Yes, gill surface area explained some of the variation in brain mass after accounting for body mass, with individuals with a large brain for their body mass also having a large gill surface area (Figure 2.1C). The model including both gill surface area and body mass as explanatory variables (AICc = -58.8, w_i = 0.78) fit the data slightly better than the model with just body mass (AICc = -56.3, w_i = 0.22) and had a greater weight of evidence out of the two candidate models.

2.5. Discussion

We found that the allometric slope of brain mass was lower than the allometric slope of gill surface area across blacktip shark individuals, and that individuals with a large brain for their body mass also have a larger gill surface area, as predicted. Next, we discuss (1) our results on the allometric relationships of brain mass and gill surface area in the context of the scientific literature, (2) our findings as they pertain to the

interrelationships between brains, gills, and energy demand, and (3) caveats of the study that should be considered and future directions of research.

Both brain mass and gill surface area increased ontogenetically with body mass, but the rate that brain mass changed with body mass (slope = 0.45) was lower than the rate that gill surface area changed with body mass (slope = 0.90). These allometric slopes show that larger blacktip shark individuals possess both larger brains and larger gill surface areas compared to smaller individuals, corroborating our predictions and the results of studies in other species (Chapman and Hulen 2001, Lisney et al. 2017, Bigman et al. 2018). Brain mass likely changes with body mass to allow for neural control, sensation, and regulation associated with a growing body (Leonard et al. 1978, Ngwenya et al. 2013). Since fishes (like other ectothermic vertebrates) grow indeterminately, their brains may exhibit lifelong neurogenesis and brain growth to match the neural demands of the body (Leonard et al. 1978, Zupanc 2006, Ngwenya et al. 2013). For example, our estimate of the brain mass allometric slope was similar to slope estimates reported in various other intraspecific studies of fishes, which reported slopes of 0.48–0.57 (Bauchot et al. 1976) and 0.5 (95% CI: 0.46–0.54; Gonda et al. 2011) for bony fishes and 0.427 (95% CI: 0.374–0.480; Lisney et al. 2017) and 0.46 (95% CI: 0.43–0.49; Laforest et al*.* in press) for cartilaginous fishes. Our slope estimate was also similar to interspecific studies across cartilaginous fishes broadly (slope = 0.43; Yopak et al. 2019) and Carcharhiniformes specifically, where brain mass changes with body mass with a slope of 0.52 (Myagkov 1991). However, this slope estimate was much lower than geometric expectations (slope = 1), or predictions based upon either body surface area (slope = $^{2}/_{3}$) or an exact match with metabolic scaling (slope = ¾, Jerison 1973 in Harvey and Bennett 1983, Armstrong 1983, Hofman 1983). Additionally, as gill surface area is a metabolically important trait that supports the oxygen diffusion necessary for aerobic metabolism in fishes, it fits that gill surface area would also change with body mass throughout ontogeny to support the energetic requirements of a larger body and potentially the energetic requirements of a growing brain (Hughes 1978, Bigman et al. 2018). The allometric slope of gill surface area found in this study matches previous work on gills that have found allometric slopes between $^{2}/_{3}$ and 1 (Wegner et al. 2010, Bigman et al. 2018), as well as the allometric slope of metabolic rate (average slope = 0.89, Jerde et al. 2019). Although our results are only correlational, the lower allometric slope of brain mass compared to gill surface area supports our prediction that gill

surface area, and by extension oxygen supply capacity, may set an upper limit for the allometric slope of brain mass (Karbowski 2007). If brain mass increased with body mass faster than gill surface area did, then individuals would potentially reach a point where they had more neural tissue than they could supply oxygen to and energetically support. These individuals would therefore be selected against. Thus, examining the ontogenetic allometries of brain mass and gill surface area provides some insight into the evolution of brain size.

Our results also suggest that the ability to uptake oxygen necessary for metabolism explains some of the variation in brain size in this species. Blacktip shark individuals with a large gill surface area for their body mass may be able to support a higher energetic turnover, and, in turn, a larger brain for their body mass. However, brain size is highly variable at large body sizes, so although our results suggest that individuals with a large brain for a given body mass may also have a large gill surface area, more data is needed spanning the full range of body size in blacktip sharks (Laforest et al. in press). Our results are consistent with the direct metabolic constraints hypothesis which suggests that the cost of evolving a larger brain can be met through the evolution of increased energy intake (Armstrong 1983, Hofman 1983, Isler and van Schaik 2009). However, this strategy is likely to be risky given that individuals would be more vulnerable to unexpected shortages of energy supply (Deaner et al. 2003, Isler and van Schaik 2009). Evidence for the direct metabolic constraints hypothesis has not previously been presented within wild individuals of a species, since studies usually use interspecific trends or laboratory experiments to demonstrate a connection between metabolism and brain size (Isler and van Schaik 2006, Kotrschal et al. 2013, Iglesias et al. 2015).

Our results show the relationship between brain mass and gill surface area, a metabolically important morphological trait that may portray a more integrated estimate of oxygen demand and energy use compared to shorter-term measures like metabolic rate (Bigman et al. submitted, Gillooly et al. 2016). However, the energetic requirements of maintaining a large brain could also require compromises that affect other organs or processes in addition to an increase in energy turnover (Aiello and Wheeler 1995, Isler and van Schaik 2009). Indeed, brain size has been shown to trade off with other energetically expensive organs or activities that are beyond the scope of this study. For example, across species, brain size has been negatively correlated with gut lengths in

fishes and anurans (Kotrschal et al. 2013, Tsuboi et al. 2015, Liao et al. 2016), gonad size in bats (Pitnick et al. 2006), and pectoral muscle size in birds (Isler and van Schaik 2006). Additionally, interspecific analyses of sharks, cichlids, and frogs, respectively, reveal that species with 'slower' life histories tend to have a larger brain for their body size (Mull et al. 2011, Tsuboi et al. 2015, Yu et al. 2018). Furthermore, an intraspecific study of guppies revealed that individuals with larger brains for their body size have fewer offspring (Kotrschal et al. 2013). Accounting for whether trade-offs occur with other energetically expensive organs or activities will strengthen our understanding of the energetic basis of variation in brain size. Hence, investigating the energetic 'budgets' (i.e., energy uptake, allocation, and use) of the blacktip shark would be an interesting avenue for future study.

The links between energy demand and oxygen availability, and between brains and gills specifically, provide a rich area for future research. Firstly, investigating the allometric relationships of separate regions of the brain – and not just total brain mass – may provide further clues about the various pressures acting on these metabolically important organs and may be particularly important for fishes, since fishes exhibit lifelong neurogenesis. For instance, individuals from marine populations of nine-spined sticklebacks (*Pungitius pungitius*) had a larger olfactory bulb and telencephalon (after accounting for both body size and total brain size) compared to individuals from pond populations, likely due to differences in habitat complexity between the environments (Gonda et al. 2009). Additionally, ontogenetic shifts in the sizes of major brain regions compared to the whole brain in the bluespotted stingray (*Neotrygon kuhlii*) and pouched lamprey (*Geotria australis*) have been associated with shifts in diet, sensory specialization, habitat use, and activity patterns (Lisney et al. 2007, 2017, Salas et al. 2015). Ontogenetic studies that focus on various brain regions may explain why we found a difference in how brain mass changed with body mass between the two sampling locations, and whether this brain growth is homogenous across all brain regions or due to hyperallometry of certain structures. Secondly, since this is a correlative study comparing brain mass and gill surface area, there may be other factors affecting these traits that we were unable to account for, like the cost of other potentially energetically expensive organs and processes such as the gut, liver, and reproductive investment. Further studies that are able to combine both *in situ* population studies and experimental manipulations that specifically include multiple generations (i.e., selection

experiments) and other expensive organs could both control for factors not investigated here and also help tease apart phenotypic plasticity from adaptive selection, a central challenge in evolutionary biology and ecology. For instance, Crispo and Chapman (2010) conducted a laboratory-rearing experiment using broods from multiple populations of an African cichlid fish (*Pseudocrenilabrus multicolor*) under high- and low-oxygen treatment. They found that variation in brain mass and gill surface area was partially due to plasticity, such that individuals in the low-oxygen treatment had smaller brains and larger gills for their body size than those in the in the high-oxygen treatment, regardless of the population that they originally came from. However, they also found population variation in brain mass within treatments, suggesting that there are also genetic effects on *P. multicolor* brain mass (Crispo and Chapman 2010).

As ectothermic metabolic rate, and thus oxygen demand, increases with temperature, future studies could also investigate the interrelationships between temperature, oxygen availability, and metabolically important traits like gill surface area and brain mass. The Gulf of Mexico and Atlantic Bight differ environmentally, with the Gulf of Mexico tending to have warmer, more oxygen-stressed waters (Rabalais and Turner 2001, Belkin 2009). Additionally, blacktip sharks from the Gulf of Mexico and Atlantic Bight are considered to be two separate populations as they are genetically and geographically distinct, with individuals from the Gulf of Mexico tending to mature earlier and grow to a smaller size (Keeney et al. 2003, 2005, Carlson et al. 2006). Studies on the differences in metabolically important traits like gill surface area and brain mass between individuals from these two populations could thus help identify the bases for temperature-body-size patterns and further our understanding of how temperature and oxygen availability may affect metabolically important morphological traits. Comparisons of traits among distinct populations fill an important gap between large-scale, interspecific comparisons across species and studies on individual variation, thus helping to improve our understanding of the evolution, and functional significance, of variation in key traits (Gonda et al. 2013). Insight into these interrelationships could also shed light on drivers of life history variation and explanations for temperature-body-size patterns.

In conclusion, our results indicate that both brain mass and gill surface area increase allometrically with body mass in the blacktip shark throughout ontogeny, but that brain mass changes with body mass at a lower rate than gill surface area does.

Additionally, our results suggest that blacktip shark individuals with larger brain masses for their body mass may also have larger gill surface areas. As with many other organismal traits, brain size is likely the result of multiple trade-offs between energetic costs and benefits. By showing that both brain mass and gill surface area increase with body mass, and that one measure of the capacity for oxygen diffusion (i.e., gill surface area) explains some of the variation in brain mass throughout ontogeny, this study provides clues as to one mechanism through which fishes may have evolved and maintained large brains, despite their high energetic cost. This work on the relationships between these two metabolically important traits as well as future studies on how allometric relationships vary between and among populations, could provide clues about the evolution of brain size and could also help us predict the effects on fishes of future challenges like increasing water temperature or severe hypoxic events, which may change energetic demands and oxygen availabilities.

2.6. Tables

Table 2.1. **Sample size (n), numbers of each sex (female, F, and male, M), and ranges of body mass (g), fork length (FL, cm), brain mass (g) and gill surface area (GSA, cm^2) for blacktip shark, *Carcharhinus limbatus*, samples for which we measured brain mass, gill surface area, or both.**

Traits measured	n	Sex	Body mass range (g)	FL range (cm)	Brain mass range (g)	GSA range (cm^2)
Brain mass	24	14 F, 10 M	750 – 12,977	45.5 – 101.0	6.02 – 21.23	
Gill surface area	19	12 F, 7 M	750 – 30,043	45.5 – 133.0		2,462.84 – 58,205.11
Both	15	10 F, 5 M	750 – 12,977	45.5 – 101.0	6.02 – 21.23	2,462.84 – 34,919.63

Table 2.2. **Coefficients of the linear regressions for brain mass and body mass and gill surface area and body mass for blacktip sharks, *Carcharhinus limbatus*. Values in brackets are the 95% confidence intervals and standardized intercepts are the back-transformed estimates of gill surface area or brain mass for a 2,000 g individual. All variables were log_{10}-transformed.**

Model	Allometric slope	Standardized intercept
brain mass ~ body mass	0.45 (0.41 – 0.49)	8.41 (8.12 – 8.71) g
gill surface area ~ body mass	0.90 (0.81 – 0.99)	5,283.82 (4,705.57 – 5,933.13) cm^2

2.7. Figures

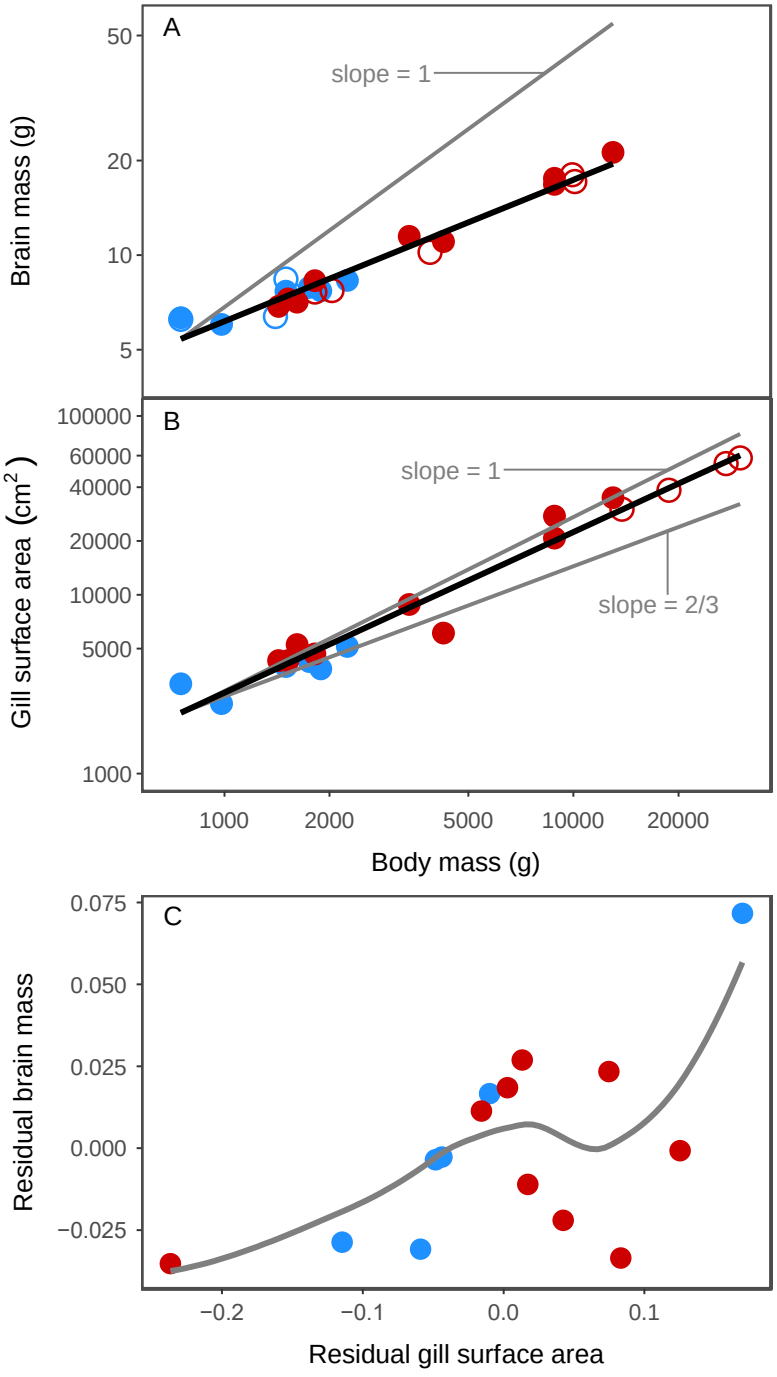
A
slope = 1
Brain mass (g)
50
20
10
5
B
slope = 1
slope = 2/3
Gill surface area (cm^2)
100000
60000
40000
20000
10000
5000
1000
1000
2000
5000
10000
20000
Body mass (g)
C
Residual brain mass
0.075
0.050
0.025
0.000
−0.025
−0.2
−0.1
0.0
0.1
Residual gill surface area

Figure 2.1. **The relationship of (A) brain mass (g) and (B) gill surface area (cm^2) to body mass (g) for blacktip shark, *Carcharhinus limbatus*, samples from the Gulf of Mexico (red) and Atlantic Bight (blue). Filled points represent individuals for which both brain mass and gill surface area were measured. The black fitted regression lines are from linear models of log_{10}-transformed gill surface area or log_{10}-transformed brain mass data as functions of log_{10}-transformed body mass. Shaded grey regions indicate the 95% confidence intervals. Grey lines depict theoretical allometric relationships with slopes of 1 (A), and 1 or 2/3 (B). (C) depicts the relationship between residual brain mass (after correcting for body mass) and residual gill surface area with a loess line to illustrate trend.**

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Chapter 3.

The metabolic pace of life histories across fishes

3.1. Abstract

All life acquires energy through metabolic processes and that energy is subsequently allocated to life-sustaining functions such as survival, growth, and reproduction. Thus, it has long been assumed that metabolic rate is related to the life history of an organism. Indeed, metabolic rate is commonly believed to set the pace of life by determining where an organism is situated along a fast-slow life history continuum. However, empirical evidence of a relationship between metabolic rate and life histories is lacking, especially for ectothermic organisms. Here, we ask whether three life history traits – maximum body mass, generation length, and growth performance – explain variation in resting metabolic rate (RMR) across fishes. We found that growth performance, which accounts for the trade-off between growth rate and maximum body size, explained variation in RMR, yet maximum body mass and generation length did not. Our results suggest that measures of life history that encompass trade-offs between life history traits, rather than traits in isolation, explain variation in RMR across fishes. Ultimately, understanding the relationship between metabolic rate and life history is crucial to metabolic ecology and has the potential to improve prediction of the ecological risk of data-poor species.

3.2. Introduction

Metabolism is the process by which all living organisms turn external resources into available energy and, in turn, allocate this energy among competing life history processes, such as survival, growth, and reproduction (Reynolds 2003, Sibly 2012, Clarke 2017). One theory, the Metabolic Theory of Ecology (MTE), proposes a mechanistic basis for understanding why metabolic rate scales with body mass with an exponent of three quarters (0.75) (Brown et al. 2004). From this, the MTE derives quarter-power scaling relationships for numerous ecological phenomena including life history traits (Gillooly et al. 2002, Brown et al. 2004). The MTE thus assumes that metabolic rate can be used as a predictive tool to understand traditionally difficult-to-

measure ecological phenomena based upon the similarity of scaling exponents. However, these predictions of many higher-order ecological phenomena are based on the underlying assumption that metabolic rate underpins life history. Surprisingly, there are few empirical tests of whether life histories are directly related to metabolic rate, particularly for ectotherms (White and Seymour 2004, Lovegrove 2009, Ton and Martin 2016). If this putative relationship between metabolic rate and life histories exists, the idea that there is an organismal physiological basis underlying conservation and global-change-related phenomena, such as overfishing, climate change responses, and extinction risk, may prove to be a reality (Hutchings 2002, Reynolds et al. 2005, Sunday et al. 2011). Thus, exploring the connections between metabolic rate and life histories may increase our understanding of the diversity of life histories and offer simple, trait-based approaches to support the development of ecological risk analyses (Reynolds 2003, Thygesen et al. 2005).

Life history traits are optimized through natural selection to maximize fitness (Hutchings 2002, Reynolds 2003, Sibly 2012). Trade-offs among life history traits arise as individuals have finite resources to allocate to the competing processes related to survival, growth, and reproduction (Reynolds 2003, Healy et al. 2019, Stearns 1989). For example, there is a trade-off between maximum size and growth rate whereby fishes either grow fast to a small size or grow slower to a larger size (Jennings et al. 1999, Juan-Jordá et al. 2013). In turn, these trade-offs and the environment provide the framework for the evolution of life history traits (Hutchings 2002, Reynolds 2003). Specifically, in response to selection imposed by a particular environment, suites of life history traits commonly co-evolve, clustering together along a fast-slow axis, with organisms that grow slower, mature later, live longer, and have a larger maximum body size on the ‘slow’ end of the continuum, and organisms with the opposite suite of traits on the fast end (Reynolds 2003, Bielby et al. 2007, Juan-Jordá et al. 2015). Thus, life history traits can characterize an organism’s pace of life, as they describe where an organism is situated along this fast-slow continuum of life history (Reynolds 2003, Healy et al. 2019, Stearns 1989). The MTE predicts that metabolic rate sets this pace of life, thus determining where organisms sit on the fast-slow continuum. Under this theory, organisms with a higher metabolic rate will sit towards the ‘faster’ end of the life history continuum, since allocation of resources to growth and reproduction is powered by a faster metabolism (Brown et al. 2004). Yet, the relationships between metabolic rate and

life histories have rarely been examined across species, and when they have, it has yielded conflicting results. For endotherms (birds and mammals), it is still unclear whether age-related life history traits such as age at first reproduction and maximum age are related to metabolic rate, even after controlling for body mass and evolutionary history (White and Seymour 2004, Furness and Speakman 2008, Lovegrove 2009). Conflicting results have also been found in studies of growth rate. While growth rate has been found to be a strong, positive predictor of resting metabolic rate across vertebrates and has also been found to positively correlate with metabolic rate in nestling songbirds, in other studies of birds and mammals no relationship has been found between growth rate and metabolic rate (Trevelyan et al. 1990, Lovegrove 2009, Ton and Martin 2016, Grady et al. 2014). Furthermore, we know little about relationships between metabolic rate and life histories for ectotherms. Fishes present a unique opportunity to examine this relationship, as they are the most speciose group of ectotherms, constitute one of the most taxonomically and metabolically diverse radiations of vertebrates, and exhibit a wide range of life histories (Killen et al. 2016, Stein et al. 2018, Rabosky et al. 2018). Thus, examining whether metabolic rate and life history traits are related across fishes allows us to test a fundamental premise of metabolic ecology in ectotherms.

Here, we ask whether life history traits explain variation in metabolic rate across fishes, after accounting for shared evolutionary history and the effects of body mass and temperature. Specifically, we examined whether three life history traits – maximum body mass, generation length, and growth performance – were related to resting metabolic rate (RMR) across 104 fish species using a phylogenetic generalized least squares regression framework (Garamszegi 2014). We hypothesized that all three life history traits would explain variation in RMR, but that growth performance would explain the most variation in RMR because it encapsulates a life history trade-off (between growth and maximum size), whereas maximum body mass and generation length do not encapsulate trade-offs. Specifically, we predicted that species with a high metabolic rate for their body mass would have the characteristics of a ‘faster’ life history – a smaller maximum body mass, a shorter generation length, and a higher growth performance.

3.3. Methods

3.3.1. Metabolic rate data collation and selection

Resting metabolic rate (RMR), measurement temperature (i.e., the temperature associated with the metabolic rate measurement), and measurement body mass (i.e., the wet body mass associated with the metabolic rate measurement) were collated from the literature. For our analysis, we only used estimates of RMR from rates of oxygen consumption for post-absorptive, post-larval fishes in which oxygen uptake due to activity was mitigated. Obtaining estimates of RMR only from peer-reviewed studies allowed us to categorize the type of metabolic rate (e.g., RMR) measured in each study with a high degree of confidence and avoid propagating potentially erroneous metabolic rate estimates. We collated raw data (i.e., separate estimates for individuals of the same species) for each of the three metabolic traits, if available, although in most cases only a species' mean was published. Thus, for our analyses, we averaged raw estimates of RMR and measurement body mass at a given measurement temperature, resulting in a species-specific mean RMR, measurement temperature, and mean measurement body mass. If more than one study reported RMR for the same species, we chose only one study to include in our dataset to avoid biasing our results towards species that were represented by multiple studies, following Killen et al. (2016). To ensure that our choice of which study for a given species to include in our dataset did not affect the results, we conducted all analyses on three separate datasets (the 'sample size dataset', the 'mass dataset', and 'the temperature dataset') resulting from the following inclusion criteria: (1) based on the largest sample size, as presented in the main manuscript, (2) based on the largest average measurement body mass, to approximate maximum body size, and (3) based on which study's measurement temperature was within the natural temperature range of the species but closest to 20˚C to minimize the range of temperatures included in the dataset following Gillooly et al. (2001) and Killen et al. (2016). If a study measured RMR at multiple measurement temperatures, we also used selection criteria to determine which RMR data to include. For more detail on data collation and our selection criteria see Appendix B section B.1.1.

3.3.2. Life history data collation, selection, and aggregation at the species level

To assess if life history traits explain variation in RMR, we collated maximum body mass, generation length, and growth performance collected from peer-reviewed studies and grey literature (hereafter 'life history study') using literature searches and FishBase (Juan-Jordá et al. 2013, Froese and Pauly 2019). These life history traits were chosen because they are available for many species and are widely used to describe fishes' life histories (Jennings et al. 1999, Juan-Jordá et al. 2013, Dulvy et al. 2014). Maximum body mass was collated from the literature or derived from maximum body length using species-specific length-weight conversions (for more detail, see Appendix B.1.2.1). Generation length and growth performance are both life history traits that are calculated from multiple other life history traits (i.e., they are both 'composite' life history traits). Generation length was calculated as T_{mat} + (T_{max} - T_{mat}) * z, where T_{mat} is age at maturity, T_{max} is the maximum age recorded for the species, and z is a constant that depends on survivorship and the relative fecundity of young versus old individuals in the population (Pacifici et al. 2013, IUCN Standards and Petitions Committee 2019). We used a conservative value of z = 0.5 that is consistent with IUCN guidelines to account for the truncation of age structure in many fish populations by overfishing (Appendix B.1.2.2; Barnett et al. 2017, IUCN Standards and Petitions Committee 2019). Growth performance is a composite life history trait that allows for the comparison of growth rates across species that differ in maximum size, and thus accounts for the trade-off between growth and maximum size (Pauly and Munro 1984, Pauly 2010). Growth performance is often calculated as phi prime, $\phi' = \log_{10}(k) + 2*\log_{10}(L\infty)$, where $L\infty$, is asymptotic length, or the mean body length that individuals in the population would reach if they were to grow indefinitely, and k (year^{-1}) expresses the rate at which the asymptotic length is approached (Pauly and Munro 1984, Pauly 2010). We also calculated growth performance using another common measure, yet our analyses were largely insensitive to this choice (Appendix B.1.2.3). Finally, for 28 of the 104 fish species, not all life history traits were available and thus life history trait values from closely related species (here, 'proxy species') were used. To ensure that our results were not sensitive to the inclusion of data from proxy species, we re-ran analyses while excluding all species for which life history trait data from proxy species were used and compared results (Appendix B.1.2.4).

3.3.3. Statistical Analyses

We included a phylogenetic random effect in all models to account for phylogenetic non-independence among residuals using phylogenetic generalized least squares (PGLS) as implemented in the *caper* package (Orme et al. 2018, Garamszegi 2014). A PGLS accounts for phylogenetic signal, a measure of the extent to which closely related species resemble each other, by estimating the value of Pagel's lambda, λ, which varies between zero and one. A Pagel's lambda value of zero indicates no phylogenetic signal in the residuals and a value of one indicates phylogenetic covariance matching expectations under a Brownian motion model of evolution (i.e., complete phylogenetic dependence, Garamszegi 2014). We constructed a supertree from two sources: (1) the teleost Fish Tree of Life (Rabosky et al. 2018), and (2) a molecular phylogeny for chondrichthyans (Stein et al. 2018) using the R package *phytools* (Revell 2012). All statistical analyses were conducted in R v. 3.6.1 (R Core Team 2019).

Do life history traits explain variation in RMR across fishes?

To test whether life history traits explain variation in RMR across fishes, we parameterized and compared four models – one for each of the three life history traits (i.e., maximum body mass, generation length, or growth performance), and a 'null model'. The 'null model' included only measurement body mass and measurement temperature as explanatory variables. For each life history model, RMR was the response variable, and measurement body mass, measurement temperature, and the respective life history trait were the explanatory variables. For all models, measurement body mass was converted to grams, measurement temperature was converted to inverse temperature, 1/(temperature*K), where K = Boltzmann's constant and temperature is in Kelvin following Gillooly et al. (2001), and then standardized, and RMR was converted to watts following Grady et al. (2014). All variables, other than inverse measurement temperature and growth performance, were $\log_{10}$-transformed for all models. It should be noted that growth performance is already on a $\log_{10}$ scale by nature of its calculation. Comparisons of the four candidate models were then made using corrected Akaike information criteria (AICc), which penalizes models for their number of estimated parameters, with smaller AICc values indicating a better model fit (Burnham and Anderson 2002). Of the candidate models, the weight of evidence for any given model was measured by its Akaike weight (w_i), the relative likelihood of the model

divided by the sum of the likelihoods of all other models. Finally, as generation length and growth performance are composite life history traits, we parameterized four additional models – two with the components of generation length (i.e., age at maturity and maximum age) and two with the components of growth performance (i.e., k and $L\infty$) as explanatory variables – to ensure that no one component of these composite traits was driving the relationship with RMR.

What is the relative importance of each life history trait in explaining variation in RMR across fishes?

To assess the relative importance of maximum body mass, generation length, and growth performance in explaining variation in RMR across fishes, we fitted a model that included measurement body mass, measurement temperature, maximum body mass, generation length, and growth performance as explanatory variables (hereafter, 'global model'). Collinearity between variables was checked using variance inflation factors (VIFs) and all VIFs were less than five (Quinn and Keough 2002). All explanatory variables were centered and scaled by subtracting the mean and dividing by twice the standard deviation (hereafter 'standardized') so that effect sizes could be interpreted and compared in terms of units of standard deviations (Gelman and Hill 2007, Garamszegi 2014).

3.4. Results

Do life history traits explain variation in RMR across fishes?

Overall, we found that the only life history trait which explained variation in RMR across fishes was growth performance, which encompasses a life history trade-off (Figure 3.1A). The best overall model (AICc = 16.84, w_i = 0.989; Table B.1) described RMR as a function of measurement body mass, measurement temperature, and growth performance. Growth performance explained variation in RMR even after accounting for the effects of measurement body mass and measurement temperature (Figure 3.1B), with species with a high metabolic rate for their measurement body mass also having a high growth performance (β = 0.24, 95% confidence interval, CI: 0.12 – 0.36; Table B.2). On the other hand, the other life histories traits did not explain variation in RMR despite our prediction that species with a high metabolic rate for their measurement body mass would have a smaller maximum body mass and a shorter generation length. Specifically,

a null model with only measurement body mass and measurement temperature had similar relative support (AICc = 27.47, w_i = 0.005) to the models containing either maximum body mass (AICc = 27.80, w_i = 0.004), or generation length (AICc = 29.58, w_i = 0.002, Table B.1). Thus, maximum body mass (Figure 3.1C) did not explain variation in RMR after accounting for the effects of measurement body mass and measurement temperature (Figure 3.1D) and generation length (Figure 3.1E) did not explain variation in RMR after accounting for the effects of measurement body mass and measurement temperature (Figure 3.1F). Similarly, none of the component traits – age at maturity, maximum age, k, or $L\infty$ – used to calculate the composite traits of generation length and growth performance explained variation in RMR on their own, as the 95% CIs of their effect sizes crossed zero (Table B.2).

What is the relative importance of each life history trait in explaining variation in RMR across fishes?

Only growth performance, measurement body mass, and measurement temperature explained variation in RMR, as evidenced by their relative effects in a global model with standardized explanatory variables (Figure B.1; Gelman and Hill 2007). Growth performance had over a four-fold larger effect on RMR compared to maximum body mass, and a 34-fold larger effect on RMR compared to generation length (Figure B.1).

Sensitivity analyses

Our findings were robust to the three different data inclusion criteria (Appendix B.2, Table B.3), an additional measure of growth performance (Tables B.4, B.5), and the use of traits from related proxy species to in-fill data gaps (Table B.6). Finally, the residuals from all models had a phylogenetic signal (λ) of 0.56 or greater, indicating that including a random effect of phylogeny is necessary when examining metabolic rate across species (Table B.1).

3.5. Discussion

Our study directly tests whether life history explains variation in RMR across fishes, and our findings help reconcile the conflicting results of previous work relating metabolic rate and life histories across species. We find that the relationship between

metabolic rate and life history only exists when accounting for life history trade-offs, such as the trade-off between growth rate and maximum size. While growth performance explained variation, neither maximum body mass nor generation length explained variation in RMR across fishes after accounting for measurement body mass, measurement temperature, and evolutionary history. First, we compare the relationships among various measures of life history and RMR and discuss these results in the context of life history trade-offs. Second, we consider the utility of this and other studies for explaining broad life history patterns and the implications for metabolic ecology. Finally, we highlight future directions for furthering our understanding of the relationships between metabolic rate and life histories.

We found that of the life history traits examined, only growth performance explained variation in RMR across fishes. We hypothesized that growth performance would explain this variation because it incorporates a trade-off between life history traits (i.e., between maximum size, $L\infty$, and growth rate, k) and thus may better characterize a fishes' life history strategy (Pauly 1981, 2010, Juan-Jordá et al. 2013). In contrast, when the components of growth performance (k and $L\infty$) were examined in isolation, they did not explain variation in RMR, emphasizing the need to examine composite indices that encompass trade-offs when investigating the relationship between RMR and life history. Although all life history traits are likely correlated due to trade-offs between them, these relationships must be explicitly captured in models, and are not captured when a single life history trait is studied in isolation (Horswill et al. 2019). However, some composite traits may not fully capture life history trade-offs among competing processes. For example, generation length is also a composite measure of life history that combines age at maturity and maximum age, yet it did not explain variation in RMR, likely because it does not capture a life history trade-off. As age at maturity increases, so does maximum age, so there is a positive, rather than negative relationship between these components of generation length which does not capture the fact that organisms that mature earlier are potentially reducing their future growth and thus body size and fecundity (Hutchings 2002, Juan-Jordá et al. 2013). The lack of relationship between RMR and maximum body mass in our study was also notable because maximum body mass is widely used as an indicator of an organism's position along the fast-slow life history continuum and is often used in assessments of extinction risk in ectothermic species (Juan-Jordá et al. 2013, Dulvy et al. 2014). Instead, the size-dependency of

metabolic rate may be mostly captured by measurement body mass, leaving little variation to be explained by maximum body mass, despite the differences in these two measures. Consequently, empirical tests of the foundations of the MTE should explicitly consider life history trade-offs in fishes, and potentially other ectotherms, rather than individual life history traits in isolation.

Testing the assumption that metabolic rate sets the pace of life histories provides insight into broad life history patterns, such as the temperature-size rule, and is a first step before using the MTE in its intended predictive capacity. Like metabolic rate, life history traits such as growth rate and maximum size are also temperature-dependent and there is a large body of literature connecting environmental temperature to growth and body size (Angilletta et al. 2004, Forster et al. 2012). This phenomenon, where individuals grow faster but attain a smaller body size at higher temperatures both in the wild (i.e., latitudinal gradients in body size and growth) and in the laboratory, has come to be known as the temperature-size rule (Angilletta et al. 2004, Forster et al. 2012). While the mechanistic basis of this phenomenon remains unresolved, hypotheses that connect metabolic processes to life history patterns, such as the oxygen limitation hypothesis, have been proposed, at least for aquatic ectotherms (Pauly 2010, Forster et al. 2012). Our results underscore the links between metabolic rate and growth performance and suggest that oxygen consumption may play a role in the temperature-size rule. Additionally, a clearer understanding of whether life history explains variation in metabolic rate across taxa is necessary before the MTE can be reliably used as a predictive model of life histories. If future studies find that life histories explain variation in metabolic rate for both endotherms and ectotherms, we will then be set with the challenge of determining whether (1) metabolic rate does indeed dictate and drive life history, (2) life history drives metabolic rate, (3) metabolic rate and life history are co-adjusted with each other, affecting each other in a reciprocal manner, or (4) both life history and metabolism are indirectly related to additional factors (Glazier 2015). These studies will not only require correlative approaches as executed here, but selection and common-garden experiments to uncover mechanistic drivers.

Other measures of metabolic rate, life history trade-offs, and statistical approaches may help clarify the relationship between metabolic rate and life history in the future. First, RMR, while the most commonly reported measure of metabolic rate, only reflects energy use and availability at rest, and does not describe the scope for

processes such as activity, growth, or reproduction (Clarke 2017). Field metabolic rate, for example, is likely a more accurate measure of day-to-day energy expenditure than RMR and thus could be more closely linked to life history strategy than RMR (Clarke 2017). Second, while our results indicate that a measure of life history that accounts for a trade-off explains variation in RMR, there are other life history trade-offs, popularised as Beverton's dimensionless ratios or Charnov's life history invariants (Gislason et al. 2010, Charnov et al. 2013). For example, natural mortality rate (M) has been found to be positively related to k from the von Bertalanffy growth function and negatively related to age at maturity, so testing whether invariants such as M/k and $T_{mat}*M$ also explain variation in metabolic rate may be a fruitful avenue for future research, especially in taxa for which reliable estimates of mortality rate are available (e.g., phytoplankton or birds; Gislason et al. 2010, Charnov et al. 2013). Third, new statistical approaches that explicitly account for trade-offs and correlations between life history traits may help us reconstruct life history strategies for species and populations that are data-poor by estimating difficult-to-measure life history traits, such as fecundity (Thorson et al. 2017, Horswill et al. 2019).

Environmental and ecological factors such as activity levels, predation risk, food availability, and environmental temperature may obscure relationships between metabolic rate and life history traits, particularly in ectotherms, and this could also be considered in the future (Forster et al. 2012, Killen et al. 2016, Bigman et al. 2018). Fish species with a high metabolic rate for their body mass have a high growth performance, but they may also have high activity levels (Pauly 2010, Killen et al. 2016). For example, Japanese amberjack (*Seriola quinqueradiata*) had a higher RMR than zander (*Sander lucioperca*), though metabolic rates of both species were measured on individuals of similar body masses and at similar measurement temperatures (Figure 3.1A). This difference in RMR may be because Japanese amberjack had a higher growth performance than zander, but activity level may also play a role. Metabolic rate is commonly used as a proxy for activity level, confounding studies of the relationship between metabolic rate and activity level (De Jager and Dekkers 1975). Thus, future studies should investigate the interrelationships between activity level, metabolic rate, and life history by using morphological proxies of activity such as the caudal fin aspect ratio (= [*height of the caudal fin*]^2/[*surface area of the fin*]; Killen et al. 2016, Bigman et al. 2018). For example, the caudal fin morphology of Japanese amberjack is strongly

lunate, suggesting that this species is more active compared to zander with its rounded tail (Figure 3.1A). Additionally, predation risk, environmental stability, and food availability, while sometimes experimentally tractable, are difficult to tease apart, let alone account for in macroecological analyses, despite likely influencing both metabolic rate and life history. However, if realistic approximations of predation risk can be attained, dynamic state variable models may provide an avenue for future investigation by featuring the trade-offs associated with life history and factors such as predation risk and food availability within a dynamic behavioural context to determine fitness (Thygesen et al. 2005). Finally, while measurement temperature greatly affects metabolic rate, environmental temperature may have an evolutionary effect on both metabolic rate and life histories through thermal constraints on production or thermal effects on survival as illustrated by broad patterns such as the temperature-size rule (Gillooly et al. 2001, Angilletta et al. 2004).

In conclusion, our analyses show that growth performance, but not maximum body mass or generation length, explains variation in RMR across a diverse set of 104 fish species. To our knowledge, this is the most comprehensive study to-date that tests whether empirical measures of life history explain variation in metabolic rate across fishes. Our findings revealed that a measure of life history that incorporates a trade-off between life history traits is strongly associated with RMR and therefore provides some support for the assumption that metabolic rate sets the pace of life across species. Insight into the links between physiology and life histories has the potential to inform ecological risk assessments, particularly for data-poor species, because life histories are closely related to risk of overfishing and extinction risk (Jennings et al. 1999, Dulvy et al. 2014, Juan-Jordá et al. 2015).

3.6. Figures

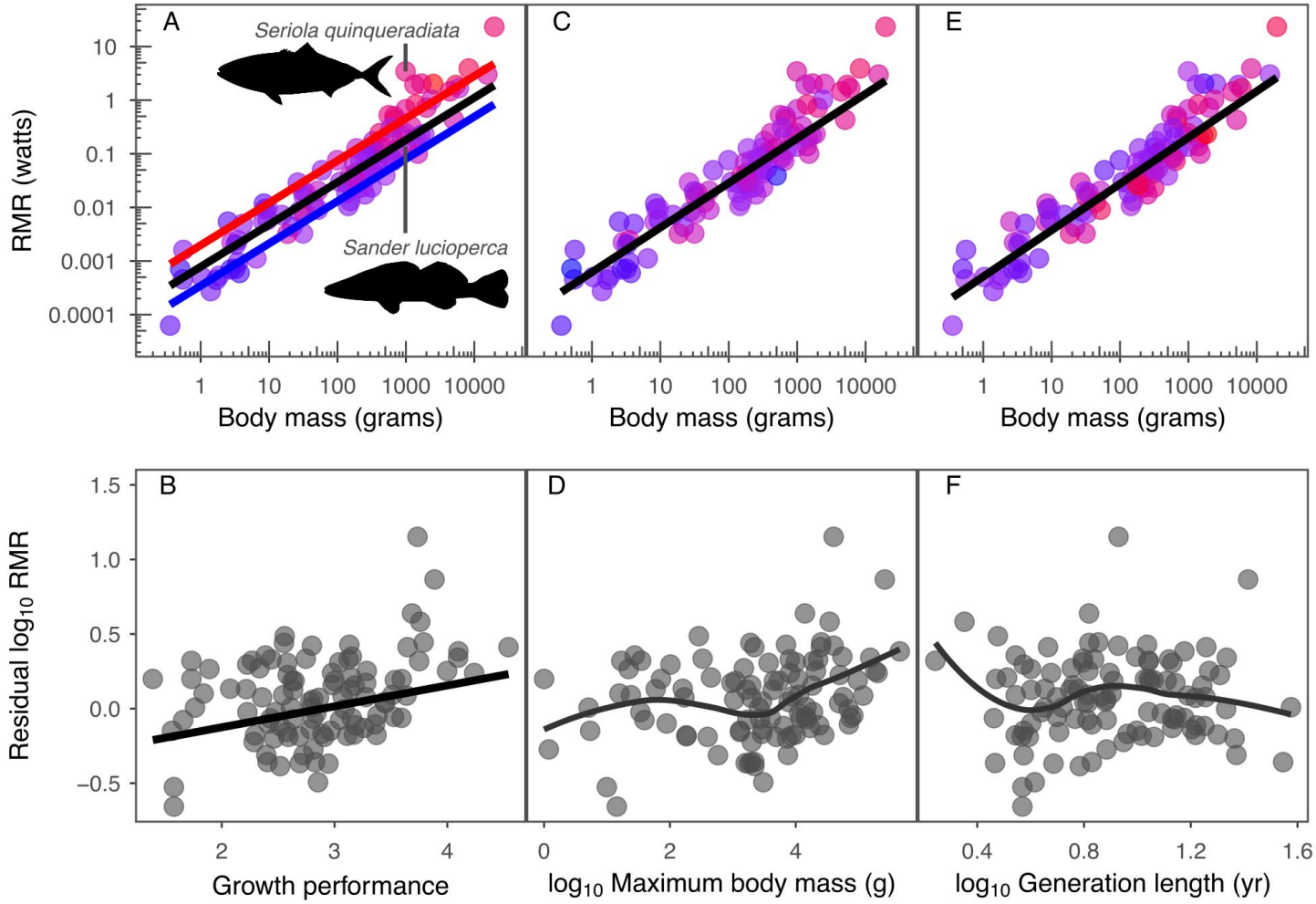
A
Seriola quinqueradiata
Sander lucioperca
C
E
RMR (watts)
10
1
0.1
0.01
0.001
0.0001
1
10
100
1000
10000
Body mass (grams)
B
D
F
Residual log10 RMR
1.5
1.0
0.5
0.0
−0.5
2
3
4
Growth performance
0
2
4
log10 Maximum body mass (g)
0.4
0.8
1.2
1.6
log10 Generation length (yr)

Figure 3.1. **Relationships between resting metabolic rate (RMR), measurement body mass, and life history across fishes. Points are coloured by growth performance (A), maximum body mass (C), and generation length (E), where red denotes larger values and blue denotes smaller values. The black fitted regression line in all three panels is the estimated RMR across the body sizes of all species in the dataset, while incorporating temperature, evolutionary history, and the relevant life history trait (maximum body mass, generation length, or growth performance). Growth performance was the only life history trait to explain variation in RMR, as illustrated by the red and blue lines in (A) which show the estimated RMR for species with high (e.g. Japanese amberjack, *Seriola quinqueradiata*) and low (e.g. zander, *Sander lucioperca*) values of growth performance while accounting for temperature and evolutionary history. The bottom row shows residual RMR after accounting for measurement body mass and measurement temperature with linear model (B) and loess (D, F) lines.**

3.7. References

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Chapter 4.

General Discussion

In this thesis, I used a combination of dissection and datamining to examine the links between metabolic rate, morphology, and life histories, with an ultimate aim of better understanding how metabolism underlies morphology and life history. In Chapter 2, I asked how brain size related to metabolic rate in the blacktip shark (*Carcharhinus limbatus*) using gill surface area as a morphological correlate of metabolic rate. In Chapter 3, I asked whether life histories explain variation in metabolic rate across fish species using the first meta-analysis of metabolic rate and empirical measures of life history in fishes. Here, I review the key findings of these studies and discuss their implications and future directions for metabolic ecology.

In Chapter 2, I found that the rate at which blacktip shark brain mass changed with body mass was lower than the rate that gill surface area changed with body mass and that individuals with a large brain for their body mass also have a large gill surface area. Though studies of other fish species have measured gills and brains from the same individuals, this is the first study to have paired samples from a shark species and is the first to test whether individuals with a large brain also have a large gill surface area for their size. These results suggest that the ability to uptake the oxygen necessary for metabolism over the gill surface area matches the energetic demands of a large brain. These findings are consistent with the direct metabolic constraints hypothesis which suggests that the costs of evolving a larger brain can be met through increased energy intake (Armstrong 1983, Hofman 1983, Isler and van Schaik 2009). Though previous studies have found that metabolic rate and brain size are positively correlated across species, and selection experiments in captive guppies (*Poecilia reticulata*) have found that brain size is negatively related to other energetically costly traits, this study provides evidence for the direct metabolic constraints hypothesis which has not previously been presented within wild individuals of a species (Kotrschal et al. 2013, Tsuboi et al. 2015). However, I also found that the rate that brain size changed with body mass differed between the two sampling locations (the Atlantic Bight and the Gulf of Mexico). Though such a difference in allometric slopes may simply be due to differences in the size range of individuals sampled from each location, it may also hint at biological differences

between the two populations that are worth investigating further. Blacktip sharks from the Gulf of Mexico likely experience warmer water temperatures (Belkin 2009) and lower oxygen availability (Rabalais and Turner 2001) than the geographically isolated and genetically distinct blacktip sharks from the Atlantic Bight (Keeney et al. 2003, 2005, Kohler et al. 2005, Bethea et al. 2012). Laboratory experiments have demonstrated short-term increases in metabolic rate and gill surface area with environmental variables such as increased temperature and decreased oxygen concentration, but the longer-term effects of environment on morphology are still not well understood (Carlson and Parsons 2001, Lefrançois and Claireaux 2003, Dowd et al. 2006, Sollid and Nilsson 2006). Although I tried to investigate how gill surface area and brain size vary between the two blacktip shark populations from different environments, our sampling was opportunistic and the body size range for individuals from the Atlantic Bight did not overlap sufficiently with the size range of individuals from the Gulf of Mexico to draw accurate conclusions for the differences between the two populations. Thus, future studies could investigate how environmental temperature and oxygen availability may affect the allometric relationships of gill surface area and brain size, taking advantage of natural differences among populations. This would provide a better understanding of the connections between environment and physiology and would also complement previous work on blacktip shark life histories which have shown that individuals from the Gulf of Mexico mature earlier and attain smaller body sizes than their counterparts from the Atlantic Bight (Carlson et al. 2006). As both brain size and gill surface area are metabolically important traits, they could have relationships with life history traits similar to that of metabolic rate, as demonstrated by both intraspecific and interspecific analyses on fishes which have found that species with ‘slower’ life histories tend to have larger brains for their body size (Mull et al. 2011, Kotrschal et al. 2013, Tsuboi et al. 2015). Thus, further studies of the relationships between brain size, gill surface area, and life history could provide a basis for using simple, morphological traits to predict life histories.

In Chapter 3, I found that growth performance, but not maximum body mass or generation length, explained variation in metabolic rate after accounting for measurement body mass, measurement temperature, and evolutionary history. Although previous studies have found correlations between metabolic rate and age-related traits like the components of generation length – age at maturity and maximum age – in

endotherms (White and Seymour 2004, Careau et al. 2009), and maximum body mass is widely believed to be correlated with many other life history traits (Juan-Jordá et al. 2013, Dulvy et al. 2014), our results show that growth performance explained more variation in metabolic rate across fishes than either maximum body mass or generation length. This is because growth performance encompasses the trade-off between growth rate and maximum body size and thus may better characterize fishes' life histories (Pauly and Munro 1984). These results indicate that although metabolic rate may be closely linked to life history, such a relationship may only be seen for measures of life history that account for trade-offs between life history traits, at least in fishes. Future work that explicitly accounts for these trade-offs and continues to empirically test the theoretical connections and assumptions underlying metabolic ecology will help ecologists revise and apply predictive models such as the Metabolic Theory of Ecology with higher confidence.

The results presented in this thesis demonstrate the need for an integrated view of ecology and physiology that recognizes the value of using trade-offs to shed new light upon the intricate interrelationships between metabolism, morphology, and life histories (Wikelski and Ricklefs 2001, Zera and Harshman 2001, Williams 2012). Brain size is likely influenced by the trade-off between cognitive ability and the energetic cost of brain tissue, which may be met, at least in part, by an enhanced gill surface area and the ability to uptake the oxygen necessary for metabolism. Additionally, contrary to predictions, the only measure of life history that explained variation in resting metabolic rate across fishes was growth performance which accounted for a trade-off between life history traits. As metabolic rate, morphological traits, and life history traits are all likely affected by external pressures and by internal constraints, examining the trade-offs associated with brain size and life history is crucial to our understanding of how organisms survive, grow, and reproduce in complex and changing environments (Maklakov et al. 2011, Juan-Jordá et al. 2015, Abelson 2016). A better understanding of the relationships between metabolic rate and life history and between metabolically important morphological traits and the environment may also provide insight into key questions in metabolic and macroecology such as whether metabolic rate does indeed set the pace of life histories, or why aquatic ectothermic organisms tend to be smaller in cooler environments (Brown et al. 2004, Forster et al. 2012). Future work is needed to test how metabolic rate and metabolically important traits vary with environment *in situ*,

as well as whether brain size and gill surface area are related to life history traits across fishes.

Though theoretical connections and broad patterns may help us explain our ecological surroundings, they must be corroborated with empirical tests such as those presented here. Although current theories provide convenient and simple models for understanding biological processes at large scales, a better understanding of the physiological mechanisms behind metabolic rate and life history trade-offs can elucidate the reasons behind these patterns and increase our understanding of the assumptions and exceptions of predictive models (Wikelski and Ricklefs 2001, Zera and Harshman 2001, Ricklefs and Wikelski 2002, Williams 2012). As the scientific community learns more about the physiological and environmental factors underlying morphology and life histories, these findings may be used to build higher-order corrections that can better inform estimates across taxa and biological levels from individuals to ecosystems. Being able to predict life history strategies from other, easier-to-measure traits such as brain size, gill surface area, or metabolic rate could allow vital estimates of population growth rates and extinction risk for data-poor species (Denney et al. 2002, Reynolds 2003, Brown et al. 2004). A better understanding of the physiological underpinnings of morphology and life history will enhance our mechanistic understanding of life history strategies and improve the predictive power of metabolic ecology.

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Appendix A.

Supplementary Material Chapter 2

Table A.1 **Sample size (n), numbers of each sex (female, F, and male, M), and ranges of body mass (g), fork length (FL, cm), brain mass (g) and gill surface area (GSA, cm^2) for blacktip shark, *Carcharhinus limbatus*, samples from the Gulf of Mexico and the Atlantic Bight.**

Measured trait by location	n	Sex	Body mass range (g)	FL range (cm)	Brain mass range (g)	GSA range (cm^2)
Brain mass						
Gulf of Mexico	14	7 F, 7 M	1,429 – 12,977	49.0 – 101.0	6.87 – 21.23	
Atlantic Bight	10	7 F, 3 M	750 – 2,250	45.5 – 57.9	6.02 – 8.39	
Gill surface area						
Gulf of Mexico	13	7 F, 6 M	1,429 – 30,043	49.0 – 133.0		4,279.42 – 58,205.11
Atlantic Bight	6	5 F, 1 M	750 – 2,250	45.5 – 57.9		2,462.84 – 5,145.34
Both						
Gulf of Mexico	9	5 F, 4 M	1,429 – 12,977	49.0 – 101.0	6.87 – 21.23	4,279.42 – 34,919.63
Atlantic Bight	6	5 F, 1 M	750 – 2,250	45.5 – 57.9	6.02 – 8.31	2,462.84 – 5,145.34

Table A.2 **Coefficients of the linear regressions for gill surface area and body mass and brain mass and body mass for blacktip sharks, *Carcharhinus limbatus*, from two locations. Values in brackets are the 95% confidence intervals and standardized intercepts are the back-transformed estimates of gill surface area or brain mass for a 2,000 g individual.**

Location	Allometric slope	Standardized intercept
*log_{10}(brain mass) ~ log_{10}(body mass) * location*		
Gulf of Mexico	0.50 (0.45 – 0.54)	8.07 (7.71 – 8.45) g
Atlantic Bight	0.28 (0.16 – 0.40)	8.01 (7.52 – 8.52) g
*log_{10}(gill surface area) ~ log_{10}(body mass) * location*		
Gulf of Mexico	0.89 (0.79 – 1.00)	5,418.40 (4,586.87 – 6,400.66) cm^2
Atlantic Bight	0.50 (0.06 – 0.94)	4,404.40 (3,507.88 – 5,530.05) cm^2

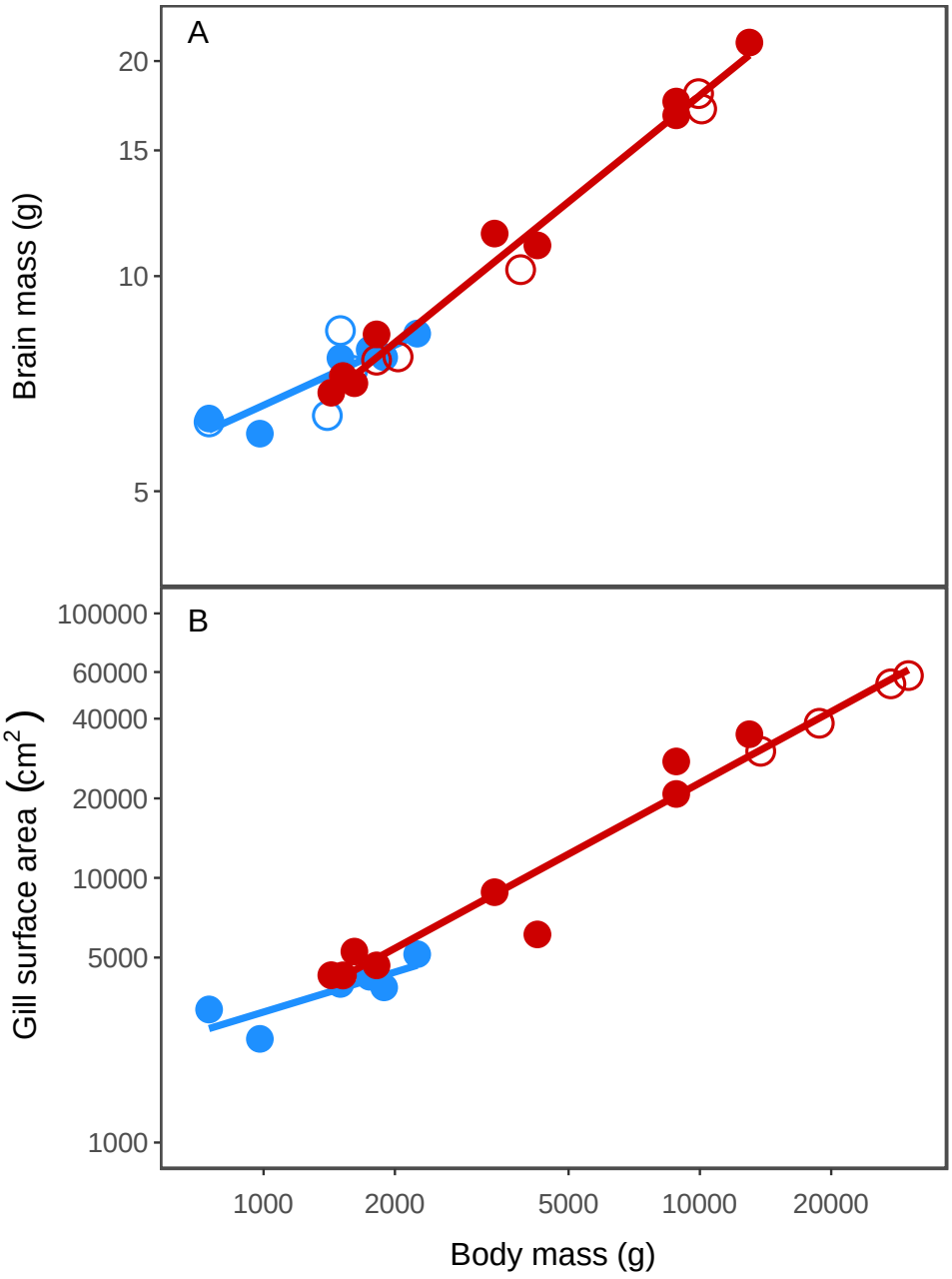

Figure A.1 **The relationship of (A) brain mass (g) and (B) gill surface area (cm^2) to body mass (g) for blacktip shark, *Carcharhinus limbatus*, samples from the Gulf of Mexico (red) and Atlantic Bight (blue). Filled points represent individuals for which both brain mass and gill surface area were measured. The fitted regression lines and equations are from linear models of log_{10}-transformed brain mass or log_{10}-transformed gill surface area data as functions of log_{10}-transformed body mass and its interaction with location. Shaded regions indicate the 95% confidence intervals.**

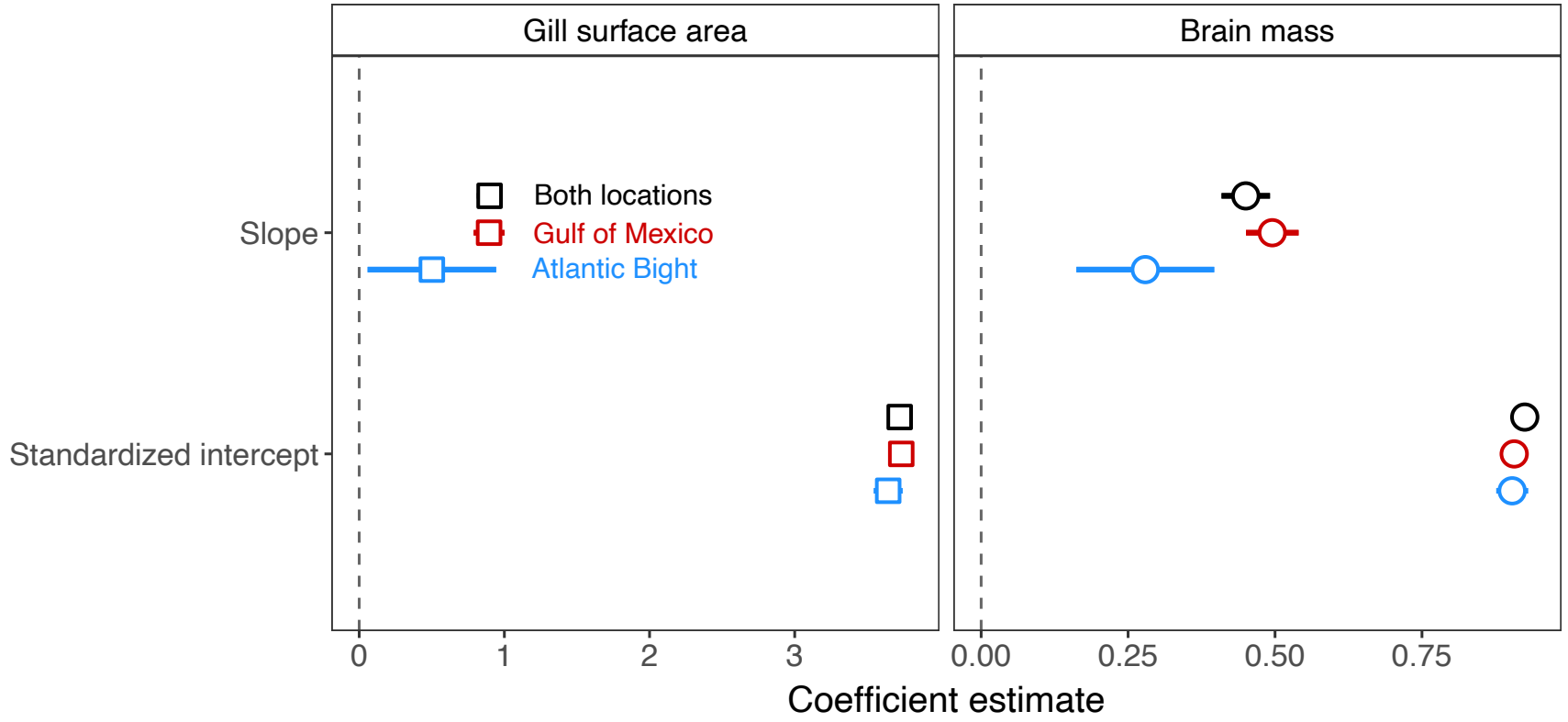

Figure A.2 **Coefficient plot showing the slopes and standardized intercepts from linear regressions of $\log_{10}$-transformed gill surface area and $\log_{10}$-transformed brain mass as functions of $\log_{10}$-transformed body mass for blacktip shark, *Carcharhinus limbatus*, samples from the Gulf of Mexico (red), Atlantic Bight (blue), and both locations combined (black). Bars represent 95% confidence intervals and the dotted line denotes a slope and intercept of 0.**

Appendix B.

Supplementary Material Chapter 3

B.1. Supplementary Methods

B.1.1. Metabolic rate data collation and selection

In most studies, resting metabolic rate (RMR) was reported as having been calculated by either extrapolating values of oxygen consumption at varying activity levels to no activity, or by measuring oxygen consumption during periods of quiescence after acclimation in the respiratory chamber. When a study reported metabolic rates for the same species at multiple temperatures, only metabolic rate measured at one temperature was included in our dataset. To choose which metabolic rate and measurement temperature to include, we used multiple inclusion criteria: (1) selecting RMR data based upon whichever measurement temperature had the largest sample size, (2) selecting RMR data based upon whichever measurement temperature had the largest average measurement body mass, and (3) selecting RMR data based upon whichever measurement temperature was closest to 20˚C, following Gillooly et al. (2001) and Killen et al. (2016). In cases where metabolic rate was measured multiple times for the same individual at the same measurement temperature, the measurement with either the largest measurement body mass or, measurement body mass being equal, the lowest RMR estimate, was used. Occasionally measurement body mass or measurement temperature were reported as ranges, in which case the midpoint was used.

B.1.2. Life history data collation, selection, and aggregation at the species level

B.1.2.1. Maximum body mass

To obtain maximum body mass for each species, we extracted the maximum observed body size (i.e., maximum body length or maximum body mass) from each life history study and from FishBase (Froese and Pauly 2019). If a range of values was given, the maximum of the range was used as it is the largest observed measurement.

To obtain a species-specific value of maximum body mass, we preferentially chose the largest value of maximum body mass provided from published papers, and if that was not available, then values from FishBase were used (Juan-Jordá et al. 2013, Froese and Pauly 2019). If values of maximum body mass from neither source were available, values of maximum body length were converted to maximum body mass using species-specific length weight regressions. For this, a length-weight regression equation from the same study that estimated maximum body length was used if it was available. If not, species-specific length-weight regression equations were obtained from FishBase (Froese and Pauly 2019). As FishBase often reported multiple length weight coefficients for each species, we took an average of these for use in converting length to weight. Each species-specific observation was documented in FishBase as having been estimated using a specific length type (e.g. fork length, standard length, etc.) from samples of all male, all female, mixed sex, or 'unsexed' individuals. Observations that were documented as either mixed sex or unsexed were all treated as mixed sex as it was not possible to know the sex composition of these samples. Mean length-weight coefficients were calculated from 'group-specific' (i.e., male, female, or mixed) data and then used to convert length to weight following the formula $W = aL^b$, where W is body mass, L is body length, a is the intercept, and b is the allometric slope.

B.1.2.2. Generation length

To estimate generation length for each species, we extracted its components – maximum age and age at maturity – from each life history study and from FishBase (Froese and Pauly 2019). We extracted the maximum observed age (empirical longevity, in years) from all life history studies in which age was estimated, as well as the theoretical longevity based on the von Bertalanffy growth function, if reported. If a range of values was given, the maximum of the range was used (Juan-Jordá et al. 2013). We compared the empirical (T_{max}) and calculated longevity ($T\infty$) estimates to evaluate their interchangeability and check for any potential errors following Juan-Jordá et al. (2013), where longevity was calculated using Taylor's relationship, $T\infty = 3/k$ (Taylor 1958). To estimate a species-specific value of maximum age, we preferentially chose the maximum measured value provided from published papers, if that was not available, then values from FishBase were used (Juan-Jordá et al. 2013, Froese and Pauly 2019). If values from neither source were available, then theoretical values of maximum age provided in peer-reviewed life history studies were used. When extracting age at

maturity, we did not differentiate between studies that estimated age at maturity as age at which 50% of the sampled individuals have matured and those that reported age at first maturity, following previous work (Juan-Jordá et al. 2013). If a range of age at maturity values was given, the midpoint of the range was used (Killen et al. 2016). To estimate a species-specific value of age at maturity, we used a simple arithmetic mean of values provided from published papers. If no values from papers were available, values from FishBase were used (Froese and Pauly 2019). We preferentially used estimates of generation length and its components for females whenever maximum age and age at maturity were reported separately for sexes (Juan-Jordá et al. 2013).

B.1.2.3. Growth performance

To estimate growth performance for each species, we mined out the $L\infty$ and k parameters from studies that estimated growth using the three-parameter formulation of the von Bertalanffy growth function (Pardo et al. 2013). We compared the maximum observed length (L_{max}) and the theoretical maximum length (i.e., asymptotic length, $L\infty$) of each species to evaluate their interchangeability and check for any potential errors following Juan-Jordá et al. (2013). We calculated growth performance using $L\infty$ and k individually for each study and then attained a single value for each species by calculating a simple arithmetic mean (giving equal weight to all the studies; Pauly 2010, Juan-Jordá et al. 2013). In order to collate estimates of L∞ across studies, we converted all $L\infty$ estimates to total lengths (TL), as other length measurements are difficult or impossible to measure for some species. Disc width (DW, the maximum width across the body) was used instead of TL for ray-like chondrichthyan fishes, as it is the standard measurement of body size for those species and estimates of TL are prone to error (Last et al. 2016). We converted other length types into cm TL (or DW for ray-like chondrichthyans) using published length-length regression equations following the same protocol as length-weight regression equations (see Appendix B.1.2.1). If the life history study provided a length-length regression equation then that was used, otherwise, mean species-specific length-length regression coefficients for each group (i.e., male, female, or mixed) and 'known length' type (e.g. fork length, standard length, etc.) were calculated from FishBase (Froese and Pauly 2019). 'Known length' was converted to TL (or DW for ray-like chondrichthyans) following the formula $TL = a + b*L$, where L is the known body length (e.g. measured as fork length, standard length, etc.), a is the intercept of the regression, and b is the slope.

We evaluated the reliability of the von Bertalanffy growth parameters of each of the species using two criteria. First, for each life history study that estimated von Bertalanffy growth parameters, we estimated the variability in the ratio between the maximum observed length (L_{max}) and asymptotic length ($L\infty$). We eliminated those studies with ratios which fell more than three standard deviations away from the mean ratio across all studies within each species (Juan-Jordá et al. 2013). Second, we examined the variability of the growth performance parameter (ϕ') calculated from each study across all studies and with species pooled. The ϕ' values for a given species or taxonomically related group of species should be normally distributed around the mean ϕ' of the taxonomic unit, and values further away from the mean of the distribution must be interpreted with increasing caution (Munro and Pauly 1983, Pauly and Munro 1984, Juan-Jordá et al. 2013). We standardized the ϕ' values of each study by dividing each by the mean of ϕ' within each species. Von Bertalanffy growth equations for which the standardized ϕ' value was greater than three standard deviations away from the mean standardized ϕ' value for all studies and species were then removed. A cut-off of three standard deviations was chosen based on previous work (Juan-Jordá et al. 2013) as well as the histogram of the standardized ϕ' (all data pooled).

Although we chose phi prime, ϕ', as a measure of growth performance because it is widely used and thus could facilitate comparisons, it has been suggested that growth performance indices based upon asymptotic weight, $W\infty$, rather than asymptotic length, $L\infty$, may be better when comparing fishes that differ in body shape (Pauly 1979, Pauly and Munro 1984, Alvarez-Lajonchère and Ibarra-Castro 2012). Thus, we re-ran analyses using a measure of growth performance based upon asymptotic weight (phi, ϕ) in order to compare results for the two measures of growth performance. Phi was calculated using the equation $\phi = \log_{10}(k) + 2/3*\log_{10}(W\infty)$ (Munro and Pauly 1983). $W\infty$ was calculated from $L\infty$ using length-weight regression equations (see Appendix B.1.2.1). As length-weight regression equations were not available for all species and groups (i.e., male, female, or mixed sex), the relationship between growth performance and RMR was investigated using a subset of data for which ϕ could be calculated. Additionally, species-specific, but not group-specific, length-weight regression equations were used to estimate $W\infty$, and thus ϕ, for a larger subset of species in a separate analysis. Thus, we tested whether weight-based growth performance explained variation in RMR using two datasets – one where ϕ was calculated from species-specific and group-specific length-

weight regression equations (n = 44) and one ϕ was calculated from species-specific but not group-specific length-weight regression equations (n = 84).

B.1.2.4. Proxy species

For the 28 species that were missing at least one life history trait, values from closely related species (i.e., 'proxy species') were mined out from life history studies of species within the same genus as the species with the missing trait value. Life history trait values from each life history study were then aggregated following the methods outlined in Appendix B.1.

B.2. Supplementary Results

Our results were similar regardless of the method of data selection used (Figure S1, Table S1-3), were generally robust to the measure of growth performance used (Table S4, S5), and were robust the inclusion of life history data from proxy species (Table S6). When growth performance was characterized using a weight-based metric (ϕ), rather than a length-based metric (ϕ'), results seemed to depend upon sample size. When ϕ was calculated using estimates of W_∞ from group- and species-specific length-weight regressions, our sample size was more than halved (n = 104 vs. n = 44). Thus, the candidate models all had similar support (all ΔAICcs were less than two and models had similar Akaike weights, w_i) and ϕ was not correlated with RMR (Table S4, S5). However, when species-specific, but not group-specific, length-weight regressions were used, we were able to obtain estimates of ϕ for a larger dataset (n = 84) and our results matched analyses using ϕ'. When models including either ϕ or ϕ' were compared with AICc, they both had similar relative support and were within two AICc of each other (Table S5).

Table B.1 **Comparisons of phylogenetic generalized least squares models investigating how life history traits explain variation in resting metabolic rate (RMR) across fishes, while accounting for measurement body mass (M_b) and measurement temperature (T). Life history traits are maximum body mass (M_{max}), generation length (GL), and growth performance (ϕ'). All variables were $\log_{10}$-transformed, and measurement temperature was modeled as standardized inverse temperature. RMR data was from either the sample size dataset (1), the mass dataset (2), or the temperature dataset (3).**

	Model: RMR ~	λ	df	AICc	ΔAICc	w_i
1) Sample size dataset						
	$M_b + T + \phi'$	0.56	4	16.84	0.00	0.99
	$M_b + T$	0.71	3	27.47	10.62	0.00
	$M_b + T + M_{max}$	0.69	4	27.80	10.96	0.00
	$M_b + T + GL$	0.72	4	29.54	12.69	0.00
2) Mass dataset						
	$M_b + T + \phi'$	0.61	4	19.35	0.00	0.99
	$M_b + T$	0.71	3	29.49	10.14	0.01
	$M_b + T + M_{max}$	0.69	4	29.93	10.58	0.00
	$M_b + T + GL$	0.71	4	31.65	12.30	0.00
3) Temperature dataset						
	$M_b + T + \phi'$	0.71	4	17.62	0.00	0.90
	$M_b + T$	0.82	3	23.13	5.51	0.06
	$M_b + T + M_{max}$	0.81	4	24.80	7.18	0.02
	$M_b + T + GL$	0.82	4	25.24	7.61	0.02

Table B.2 **Coefficients and 95% confidence intervals (CIs) from phylogenetic generalized least squares models investigating how life history traits explain variation in resting metabolic rate (RMR) across fishes (n = 104), while accounting for measurement body mass and measurement temperature. The 'null' model included only measurement body mass and measurement temperature as explanatory variables. Life history traits are maximum body mass (M_{max}), generation length (GL), growth performance (ϕ'), age at maturity (T_{mat}), maximum age (T_{max}), growth rate (k), and asymptotic length ($L\infty$). RMR data used to estimate these coefficients came from the sample size dataset. All variables were $\log_{10}$-transformed, and measurement temperature was modeled as standardized inverse temperature.**

	Intercept			Measurement body mass			Measurement temperature			Life history trait		
		95% CI			95% CI			95% CI			95% CI	
		lower	upper		lower	upper		lower	upper		lower	upper
null	-3.26	-3.63	-2.89	0.87	0.81	0.92	-0.52	-0.65	-0.39	–	–	–
M_{max}	-3.33	-3.70	-2.95	0.83	0.75	0.91	-0.52	-0.65	-0.39	0.04	-0.02	0.11
GL	-3.29	-3.71	-2.87	0.86	0.81	0.92	-0.52	-0.66	-0.39	0.03	-0.19	0.26
ϕ'	-3.76	-4.15	-3.38	0.79	0.72	0.86	-0.48	-0.60	-0.36	0.24	0.12	0.36
T_{mat}	-3.24	-3.62	-2.87	0.87	0.81	0.94	-0.51	-0.65	-0.36	-0.04	-0.22	0.15
T_{max}	-3.30	-3.73	-2.87	0.86	0.81	0.92	-0.53	-0.66	-0.39	0.04	-0.16	0.25
k	-3.19	-3.56	-2.82	0.88	0.82	0.93	-0.49	-0.62	-0.35	0.12	-0.03	0.28
$L\infty$	-3.49	-3.94	-3.03	0.83	0.76	0.91	-0.53	-0.66	-0.40	0.17	-0.05	0.38

Table B.3 **Coefficients and 95% confidence intervals (CIs) from phylogenetic generalized least squares models investigating how life history traits explain variation in resting metabolic rate (RMR) across fishes (n = 104), while accounting for measurement body mass (M_b) and measurement temperature (T). Life history traits are maximum body mass (M_{max}), generation length (GL), and growth performance (ϕ'). RMR data used to estimate these coefficients came from either the mass dataset or the temperature dataset. All variables were $\log_{10}$-transformed, and measurement temperature was modeled as standardized inverse temperature.**

	Mass dataset			Temperature dataset		
		95% CI			95% CI	
		lower	upper		lower	upper
$RMR \sim M_b + T$						
Intercept	-3.26	-3.64	-2.89	-3.25	-3.67	-2.82
M_b	0.87	0.82	0.93	0.88	0.82	0.93
T	-0.52	-0.65	-0.39	-0.41	-0.55	-0.28
$RMR \sim M_b + T + M_{max}$						
Intercept	-3.33	-3.70	-2.95	-3.28	-3.71	-2.85
M_b	0.84	0.76	0.92	0.86	0.77	0.94
T	-0.52	-0.65	-0.39	-0.41	-0.55	-0.27
M_{max}	0.04	-0.02	0.11	0.02	-0.04	0.09
$RMR \sim M_b + T + GL$						
Intercept	-3.27	-3.69	-2.85	-3.22	-3.69	-2.76
M_b	0.87	0.81	0.93	0.88	0.82	0.94
T	-0.52	-0.66	-0.38	-0.41	-0.55	-0.27
GL	0.00	-0.22	0.23	-0.03	-0.25	0.19
$RMR \sim M_b + T + \phi'$						
Intercept	-3.75	-4.15	-3.35	-3.66	-4.09	-3.22
M_b	0.80	0.73	0.87	0.80	0.73	0.88
T	-0.48	-0.60	-0.36	-0.36	-0.49	-0.23
ϕ'	0.23	0.11	0.36	0.20	0.07	0.34

Table B.4 **Coefficients and 95% confidence intervals (CIs) from phylogenetic generalized least squares models investigating whether weight-based growth performance (ϕ) explains variation in resting metabolic rate (RMR), while accounting for measurement body mass (M_b) and measurement temperature (T). Growth performance was calculated using (A) group-and species-specific length-weight regressions as well as (B) species-specific, but not group-specific, length-weight regression equations. Measurement temperature was modeled as standardized inverse temperature and all variables were $\log_{10}$-transformed.**

	A (n = 44)			B (n = 84)		
		95% CI			95% CI	
		lower	upper		lower	upper
Intercept	-3.28	-3.69	-2.88	-3.35	-3.67	-3.02
M_b	0.82	0.71	0.94	0.82	0.75	0.89
T	-0.45	-0.61	-0.28	-0.50	-0.63	-0.38
ϕ	0.09	-0.09	0.27	0.13	0.01	0.25

Table B.5 **Comparisons of phylogenetic generalized least squares models investigating how life history traits explain variation in resting metabolic rate (RMR) across fishes, while accounting for measurement body mass (M_b) and measurement temperature (T). Life history traits are maximum body mass (M_{max}), generation length (GL), length-based growth performance (ϕ'), and weight-based growth performance (ϕ). All variables were $\log_{10}$-transformed, and measurement temperature was modeled as standardized inverse temperature. Weight-based growth performance was calculated using (A) group- and species-specific length-weight regression equations as well as (B) species-specific, but not group-specific, length-weight regression equations.**

	Model: RMR ~	λ	df	AICc	ΔAICc	w_i
A (n = 44)						
	$M_b + T$	0.81	3	5.33	0.00	0.35
	$M_b + T + \phi'$	0.82	4	6.39	1.05	0.21
	$M_b + T + \phi$	0.71	4	6.77	1.44	0.17
	$M_b + T + GL$	0.78	4	6.92	1.59	0.16
	$M_b + T + M_{max}$	0.83	4	7.54	2.21	0.12
B (n = 84)						
	$M_b + T + \phi'$	0.74	4	-2.38	0.00	0.42
	$M_b + T + \phi$	0.71	4	-2.05	0.33	0.36
	$M_b + T$	0.77	3	0.01	2.39	0.13
	$M_b + T + M_{max}$	0.77	4	1.75	4.13	0.05
	$M_b + T + GL$	0.78	4	2.13	4.51	0.04

Table B.6 **Coefficients and AICc comparisons for phylogenetic generalized least squares models investigating how life history traits explain variation in resting metabolic rate (RMR) while excluding life history data from proxy species (n = 76) and while accounting for measurement body mass (M_b) and measurement temperature (T). Life history traits are growth performance (ϕ'), maximum body mass (M_{max}), and generation length (GL). RMR data for these analyses came from the sample size dataset. All variables were $\log_{10}$-transformed, and measurement temperature was modeled as standardized inverse temperature.**

	Estimate	lower 95% CI	upper 95% CI	λ	df	AICc	ΔAICc	w_i
$RMR \sim M_b + T + \phi'$				0.72	4	1.40	0.00	0.92
Intercept	-3.61	-4.04	-3.17					
M_b	0.78	0.71	0.86					
T	-0.50	-0.64	-0.36					
ϕ'	0.19	0.07	0.32					
$RMR \sim M_b + T$				0.79	3	7.95	6.55	0.03
Intercept	-3.18	-3.59	-2.78					
M_b	0.84	0.78	0.91					
T	-0.55	-0.70	-0.41					
$RMR \sim M_b + T + M_{max}$				0.79	4	8.22	6.83	0.03
Intercept	-3.26	-3.67	-2.85					
M_b	0.81	0.73	0.89					
T	-0.55	-0.70	-0.41					
M_{max}	0.04	-0.02	0.11					
$RMR \sim M_b + T + GL$				0.79	4	10.17	8.77	0.01
Intercept	-3.19	-3.64	-2.74					
M_b	0.84	0.78	0.91					
T	-0.56	-0.70	-0.41					
GL	0.01	-0.22	0.23					

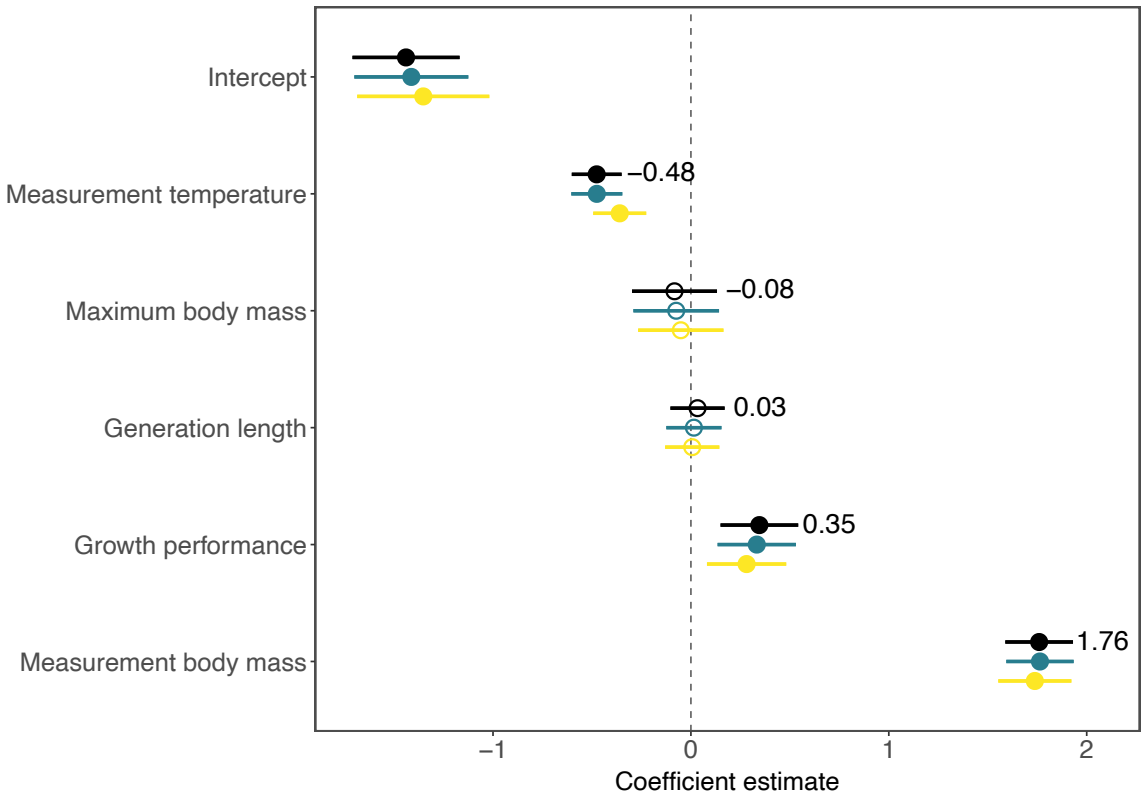

Figure B.1 **Coefficient plot of the relative effects of measurement body mass, measurement temperature, growth performance, maximum body mass, and generation length on resting metabolic rate (RMR) from global phylogenetic generalized least squares models (n = 104). Models were run using RMR data from three datasets – either the sample size dataset (black), the mass dataset (green), or the temperature dataset (yellow). Values next to the black points are the resulting model coefficients from the sample size dataset. Measurement temperature was modeled as inverse temperature, all variables except measurement temperature were $\log_{10}$-transformed, and all explanatory variables were standardized. Horizontal lines represent 95% confidence intervals.**

B.3. References

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